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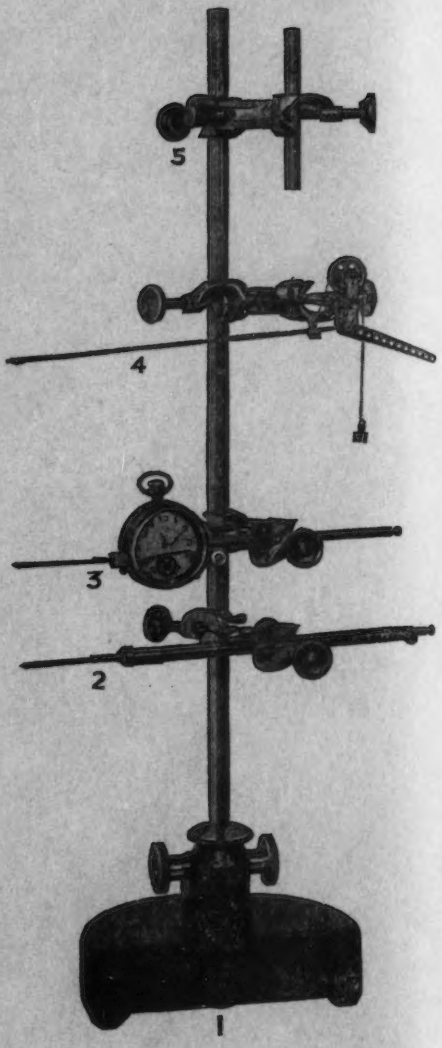

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THE COMPOSITION OF THE URINE OF STEERS AS AFFECTED BY FASTING[1]

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From the Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Mass.

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For a number of years the Nutrition Laboratory, in coöperation with the Agricultural Experiment Station of the University of New Hampshire, has been studying the total metabolism of steers. These studies have included the effect of undernutrition upon metabolism, digestion and subsequent realimentation (Benedict and Ritzman, 1923). The work has been extended to study the effect of fasting. In the conduct of these experiments the feces and urine were collected and this communication deals with the composition of the urine as affected by fasting.

The urines of two adult steers, weighing at different times from 724 to 589 kgm., were collected in periods of 24 hours or under in 7 fasts which varied in length from 4 to 14 days, and in one fast of 5 and 6 days with two young steers with weights of 248 and 277 kgm. The preceding rations were pasture, full feed, or submaintenance.[2]

PREVIOUS STUDIES OF URINES OF FASTING RUMINANTS. The earliest work on fasting steers in which the urine was collected and analyzed was by Grouven (1864) in 1861 and 1862. The weight, specific gravity, total nitrogen, dry matter, ash, and occasionally hippuric acid, were determined. Five animals were studied in 9 fasts which varied from 43½ hours to 8 days in length. Baer (1906) studied acidosis with three goats that fasted for periods of over 3 days, but, beyond the third or fourth day, phlorizin

[1] Preliminary reports of some of the results were given at a meeting of the American Society of Biological Chemists (Journ. Biol. Chem., 1923, lv, iii), and at a meeting of the National Academy of Sciences (Proc. Nat. Acad. Sci., 1925, xi, 155).

[2] For complete details of the animals, previous rations, time of last feeding, first food after fasting and daily live weights, see Benedict and Ritzman, Carnegie Institution of Washington, Publication 377, 1927.

was given. Prayon (1910) determined the per cent of creatinine in the urines of an ox, a bull, and a mare during normal and 3-day fasting periods. Peters (1920) studied the acidity (reaction), titratable alkalinity, ammonia and the chloride excretion of the goat with various diets. Blatherwick (1920) made a study of the regulation of neutrality in the blood of a cow with analyses of the blood plasma and urine during a 7-day fast. Palladin (1924a) studied the urinary creatine and creatinine with normal fasting sheep. Later (Palladin, 1924b) the rôle of ammonia formation in plant-eating animals was studied and the per cent of the total nitrogen as ammonia increased. Sjollema and van der Zande (1923) determined the total acetone bodies, ammonia nitrogen, phosphoric acid, glucose and calcium in the urine of milch cows. To provoke acetonemia they injected phlorizin followed by fasting. Following injections, the urine contained 2.2 to 2.4 per cent of glucose, but no acetone bodies. A slight ketonuria occurred only during a 2-day fast following phlorizin glycosuria. They conclude, therefore, that the cow does not easily produce much acetone, except in certain diseased conditions. Forbes, Fries and Kriss (1926) report the elimination of feces and urine by fasting cows during experiments of 3, 6 and 9 days.

METHOD OF PRESERVATION. The experimental work of the study, from which the data in this communication were drawn, was conducted at the New Hampshire Agricultural Experiment Station. The analytical work on the urines was carried out in the Nutrition Laboratory. Because of expediency, it was necessary to store the urines for several days in Durham, N. H. They were then shipped to Boston in convenient lots and subsequently analyzed. At first only nitrogen and inorganic constituents were determined. In an attempt to preserve the urine so that a more complete analysis could be made, chloroform was placed in the collecting bottles, so that as soon as urine was voided it would contain a preservative. These urines were kept in a cool place. Subsequent analyses, however, showed such a high ammonia content that it was evident that decomposition had taken place. Chloroform with thymol was next used, but this combination proved inadequate. It was then conceived that the decomposition took place not necessarily because of the effect of bacteria or of enzymes but because of the chemical reaction of the urines at the time they were voided. Since the urine of ruminants, as voided, is highly alkaline, varying amounts of concentrated hydrochloric acid were placed in the collection bottles. If, later, it was found that the urine was still alkaline, acid was added to the urine until it reacted acid. On arrival at the Nutrition Laboratory they were tested again and more acid was added, if necessary.

Since this method of preservation was begun, Brandt and Stokstad (1924) report the advantage of adding sulphuric or hydrochloric acid instead of disinfectants. They recommend a pH of 5 or 5.2 to prevent decomposition in the 24-hour urine.

METHODS OF ANALYSIS. The total nitrogen, urea nitrogen, amino-acid nitrogen,[3] creatine and creatinine,[3] were determined by methods of Folin (1922) and collaborators. The permutit method (Folin and Bell, 1917) was utilized for the determination of ammonia and gave good results with control solutions, and it is believed that it gave trustworthy results with the steers' urines which were now preserved with hydrochloric acid. The hippuric acid was determined by the method of Kingsbury and Swanson (1921). The three forms of sulphur, phosphates and total fixed bases were determined by the methods of Fiske (1921, 1922). In some of the urines, the sulphurs were also estimated by the method of Folin (1905a). The chlorides in urines not preserved with acid were determined either gravimetrically by direct precipitation with silver-nitrate solution or by the Harvey (1910) modification of the Volhard titration method applied to solutions resulting from ashing at low red heat and subsequent extraction with hot water. The acetone bodies were determined according to Folin and Denis (1914). In the determination of β-oxybutyric acid the amount of bichromate taken was such that there was always more than sufficient to oxidize the organic matter of the urine. The titratable organic acids were determined by the method of Van Slyke and Palmer (1920).

COMPOSITION OF STEERS' URINES DURING FASTING. Previous to May, 1922, the urines were collected in 24-hour periods, removing the receptacle once a day, so that the exact time of the last voiding is known to probably within a half-hour. After May 9, 1922, the periods are, for the most part, exact records as an alarm system (Benedict and Ritzman, 1927) was then used to notify the assistant that a voiding had taken place. In March, 1924, an attempt was made to separate the time into 12-hour periods. However, there are variations from this; for example, with C, the shortest period of time was 3 hours and 24 minutes and the longest period, 23 hours and 12 minutes. In tables 1 and 2 the time of fasting is included between horizontal lines. In several fasts material for analysis was obtained before the fast and also in periods following.

In the following discussion the total nitrogen, the chlorides and the sulphurs which were determined in most of the fasts will be considered first (see tables 1 and 2), and subsequently, the more complete analyses of the urines which were made only in the fasts after pasture and after submaintenance feeding will be discussed (see table 2).

ELIMINATION OF CONSTITUENTS PER HOUR. *Volume.* In order to compare the various fasts with one another, the hourly basis is used. With animals as large as these and with such varying habits in water consumption, the volumes of urine per hour varied with C from 407 cc. to 52 cc.; and with D, from 535 cc. to 46 cc.; with E, we have a very high value of

[3] The amino-acid reagent and the standard creatinine were kindly furnished by Prof. Otto Folin, Harvard Medical School, Boston, Mass.

TABLE 1

Amounts per hour of constituents determined in urines of fasting steers

FEED LEVEL, INITIAL BODY WEIGHT, ANIMAL AND DATE	DURATION OF PERIOD		VOLUME OF URINE	TOTAL NITROGEN	INORGANIC SULPHATE (S)	ETHEREAL SULPHATE (S)	NEUTRAL SULPHUR (S)	CHLORIDES (NaCl)
1921	*hrs.*	*mins.*	*cc.*	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Maintenance, 589 kgm.								
Steer C								
December 6–7	24±		269	2.70	26	44	49	1210
December 7–8	21	39	157	2.77	3	103	10	257
December 8–9	26	25	117	3.08	2	56	10	69
December 9–10	18	28	103	2.29	3	15	18	57
December 10–11	23	48	122	2.13	1	20	18	60
December 11–12	28	00	93	1.98	2	26	13	52
December 12–13	17	45	91	2.41	1	30	17	46
Maintenance, 607 kgm.								
Steer D								
December 6–7	24±		351	2.05	5	38	43	1420
December 7–8	23	46	121	2.13	1	85	4	369
December 8–9	23	45	60	2.03	30	18	6	12
December 9–10	24	30	223	2.50	3	28	12	
December 10–11	15	59*	112	2.05	1	25	8	
December 11–12	24	50	57	2.14	1	28	10	12
December 12–13	24	40	200	2.06	1	33	13	33
Maintenance, 596.6 kgm.								
1922								
Steer C								
January 4–5	24±		384	5.15	13	173	43	1790
January 5–6	24±		179	4.91	33	165	15	438
January 6–7	24	2	134	3.74	110	40	28	108
January 7–8	15	43	103	2.81	85	34	16	46
January 8–9	29±		78	2.31	62	40	11	58
January 9–10	27½±		165	2.27	74	14	30	167
January 10–11	21±		98	1.77	32	42	13	162
January 11–12	27±		59	1.61	23	40	15	33
January 12–13	18	3	86	1.68	51	33	9	97
January 13–14	27±		67	1.66	47	29	14	84
Maintenance, 611.2 kgm.								
1922								
Steer D								
January 4–5	24±		535	5.07	143	37	71	2030
January 5–6	23±		150	3.91	46	141	34	728
January 6–7	25½±		173	3.35	98	21	37	167
January 7–8	24	0	129	2.78	88	22	30	
January 8–9	22½±		138	2.53	82	25	23	
January 9–10	21½±		139	2.32	67	22	24	
January 10–11	27	55	152	1.99	53	8	25	
January 11–12	23±		80	1.64	33	15	16	
January 12–13	21½±		61	1.50	33	14	13	
January 13–14	26±		93	1.37	26	28	13	45

TABLE 1—*Continued*

FEED LEVEL, INITIAL BODY WEIGHT, ANIMAL AND DATE	DURATION OF PERIOD		VOLUME OF URINE	TOTAL NITROGEN	INORGANIC SULPHATE (S)	ETHEREAL SULPHATE (S)	NEUTRAL SULPHUR (S)	CHLORIDES (NaCl)
1922	*hrs.*	*mins.*	*cc.*	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Maintenance, 608.2 kgm.								
Steer C								
April 17–18	24±		216	3.26	21	157	18	2310
April 18–19	21±		363	3.58	43	140	16	655
April 19–20	24±		217	2.83	84	49	18	
April 20–21	24±		129	3.28	120	27	12	
April 21–22	24±		82	2.41	58	17	24	72
April 22–23	27±		140	2.37	56	32	23	44
April 23–24	21	10	101	2.03	43	20	27	44
April 24–25	24	25	77	2.00	43	28	18	40
April 25–26	23½±		86	2.23	49	22	32	24
April 26–27	20½±		101	1.67	42	17	28	52
April 27–28	30	16	151	1.71	54	17	23	179
April 28–29	22±		90	1.65	44	21	18	92
April 29–30	20±		78	1.99	52	21	19	73
April 30–May 1	30±		77	1.57	39	19	13	87
Maintenance, 624.0 kgm.								
Steer D								
April 17–18	24±		231	3.80	4	146	39	2230
April 18–19	24	22	273	3.65	1	135	24	779
April 19–20	21	34	322	3.65	38	70	28	349
April 20–21	25	22	124	2.80	52	31	17	37
April 21–22	24	37	97	2.53	55	18	33	60
April 22–23	24	16	92	2.33	51	20	30	61
April 23–24	23½±		65	1.66	34	16	20	26
April 24–25	24½±		74	1.80	35	8	25	
April 25–26	23½±		80	1.63	36	14	17	
April 26–27	25±		305	1.80	51	3	20	276
April 27–28	23	42	96	1.48	36	2	24	196
April 28–29	24	13	155	1.54	39	14	13	275
April 29–30	23	16	242	1.54	33	17	13	305
April 30–May 1	22±		85	1.54	30	16	15	96
Maintenance, 603.8 kgm.								
Steer C								
June 1–2	24±		288	4.19	78	106	42	1640
June 2–3	23	59	407	4.30	73	107	41	2100
June 3–4	22	34	176	3.57	82	68	40	492
June 4–5	23	15	105	2.67	50	44	33	133
June 5–6	25	55	125	2.54	67	38	25	167
Maintenance, 611.4 kgm.								
Steer D								
June 1–2	23±		291	4.08	19	125	52	3180
June 2–3	24	29	159	3.93	62	131	41	
June 3–4	24	11	363	3.64	78	71	42	
June 4–5	23	53	122	2.77	37	42	33	78
June 5–6†	31±		235	2.03†	16†	35†	22†	

TABLE 1—*Concluded*

FEED LEVEL, INITIAL BODY WEIGHT, ANIMAL AND DATE	DURATION OF PERIOD		VOLUME OF URINE	TOTAL NITROGEN	INORGANIC SULPHATE (S)	ETHEREAL SULPHATE (S)	NEUTRAL SULPHUR (S)	CHLORIDES (NaCl)
1923	*hrs.*	*mins.*	*cc.*	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Pasture, 704.4 kgm.								
Steer C								
November 6–7	22±		263‡	4.50‡	56‡	65‡	61‡	
November 7–8	25	40	133	3.92	73	46	23	
November 8–9	17	45	99	3.35	39	79	26	
November 9–10	29	35	97	3.19	121	12	18	
November 10–11	24	55	112	3.21	93	31	18	
November 11–12	15	35	119	3.16	89	39	21	
November 12–13	24	42	104	2.97	61	29	22	
November 13–14	24	16	105	2.74	54	16	25	
November 14–15	29±		89	2.48	54	8	29	
Pasture, 699.4 kgm.								
Steer D								
November 6–7	22±		276	4.52	90	64	32	
November 7–8	20	20	139	4.51	25	88	61	
November 8–9	27	55	97	3.34	95	8	33	
November 9–10	25	10	127	3.18	60	35	28	
November 10–11	23	35	237	3.65	93	37	27	
November 11–12	24	45	155	2.88	37	29	28	
November 12–13	16	50	109§	2.84§	41§	22§	29§	
November 13–14	23	55	81	2.28	33	20	27	

* Urine for the preceding 5½ hours on December 10 was lost.

† Steer D was fed 1760 grams of hay and 1000 grams of grain at 12:00–1:37 p.m., June 6.

‡ Some urine was spilled.

§ Approximately 200 grams of urine were lost.

642 cc. per hour for a period of 2¼ hours on February 14, 1924, and the lowest is in the next succeeding period, 46 cc. per hour for about 14 hours. Steer F shows a very narrow range of values per hour. For C and D, the lowest general average during a fast is with C, March 3 to 13, 1924.

Nitrogen. The nitrogen per hour is more dependent upon the preceding ration than it is upon the body weight. For example, the body weight of C at the beginning of the fast of March, 1924, following submaintenance, was 634 kgm. This is the third highest weight in the whole series with this steer, yet the nitrogen per hour is lower in this fast than in any of the preceding fasts and instead of a fall as in all the other fasts, there is a slight rise from the first day to the fourth day when it reached a level of approximately 1.6 grams. Similar minimum values are obtained for this same

animal in January and April, 1922. Here, however, the initial values per hour are higher than the initial value in the fast of March 3 to 13, 1924. It would appear as if 1.6 or 1.7 grams was the minimum value obtainable for an animal of this character and weight. An excretion of 1.6 grams per hour corresponds to 38.4 grams per 24 hours. If we calculate from this amount a value for a man one-tenth the weight of steer C, that is, approximately 60 kgm., we would have 3.84 grams of nitrogen per 24 hours, which is similar to the low value which was obtained by Folin (1905b) with the starch paste cream diet. His subjects were not fasting, but had a presumably adequate energy intake. In April and June, 1922, and November, 1923, with C, the level reached on the fifth day is approximately the same for all. This is in spite of the fact that the nitrogen elimination on the first day is considerably higher in June. The results with D were similar in character to those obtained with C. Feeding lowers the value from that of the last day of the fast, presumably due to the protective action of the carbohydrate in the food.

In March, 1924, the first portion of the 24-hour urine is usually collected entirely during a daylight period and the latter part is usually during the late afternoon and includes the night period. With D, the night urine is usually lower in nitrogen than the day urine. On March 7, the day value is 2.78 per hour and for the night it is 1.82. Although these values were obtained during fasting, yet the night and day difference persists even when food is given. Here one might ascribe it to the fact that the food is eaten during the daytime and the increase in nitrogen elimination is due to the nitrogen in the food.

With the younger animals E and F, the nitrogen, as with C and D during submaintenance, increased during the fast in striking contrast to the fast following pasture with maintenance feeding. With E and F at the end of the fasts it averaged 1.3 grams. With feeding there was an immediate decrease. With all four animals which fasted following submaintenance, the striking feature was the rising nitrogen as the fast progressed instead of the falling nitrogen when the animals were following pasture or maintenance feeding.

Relation of nitrogen to volume. It might be supposed that the irregularities in the values per hour were due to the incomplete excretion of the urine and that consequently there would be corresponding irregularities in the nitrogen excretion. But in spite of irregularities in volume there is a tendency to regularity in the nitrogen excretion. For example, with C the urine varies during the fast of March 3 to 13 from 140 to 52 cc. per hour. The urines from March 5 to March 13 are fairly regular in amount, but previous to that they are irregular. In spite of the variation in volume, there is a regular change in the nitrogen excretion per hour until March 5 to 6, when it reaches a fairly constant level, remaining

TABLE 2

Amounts per hour of constituents determined in urines of fasting steers

FEED LEVEL, INITIAL BODY-WEIGHT, ANIMAL, AND DATE	DURATION OF PERIOD		VOLUME OF URINE	TOTAL NITROGEN	UREA NITROGEN	AMMONIA NITROGEN	PREFORMED CREATININE	TOTAL CREATININE	AMINO ACID NITROGEN	HIPPURIC ACID NITROGEN	INORGANIC SULPHATE (S)	ETHEREAL SULPHATE (S)	NEUTRAL SULPHUR (S)	ACETONE AND DIACETIC ACID	B-OXYBUTYRIC ACID	TOTAL FIXED BASES 0.1 N	ORGANIC ACIDS 0.1 N
1923	*hrs.*	*mins.*	*cc.*	*grams*	*grams*	*grams*	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*cc.*	*cc.*
Pasture, 723.8 kgm.																	
Steer C																	
November 5–6	24±		275	3.63	2.75		0.52	0.79	38	134	40	90	34	0	tr.	1225	305
November 6–7	22±		191	4.32	3.36		0.60	1.07	21	97	29	94	65	0	7	630	230
November 7–8	24±		110	3.24	2.68		0.57	0.97	14	47	21	14	57	tr.	5	160	175
November 8–9	24	0	119	2.65	2.15		0.59	0.75	10	35	10	19	65	3	5	175	155
November 9–10	22	40	144	2.45	1.82		0.61	0.71	15	36	7	38	58	8	15	260	165
Pasture, 707.0 kgm.																	
Steer D																	
November 5–6	24±		323	3.38	2.66		0.53	0.76	67	114	38	75	86	0	13	1265	245
November 6–7	24	0	145	3.82	3.14		0.74	1.06	13	75	26	58	90	0	7	405	195
November 7–8	21	40	146	3.71	2.44		0.74	1.09	16	59	28	29	51	2	8	90	210
November 8–9	26	5	100	2.81	2.08		0.66	0.78	9	36	7	29	60	8	10	55	150
November 9–10	24±		71	2.02	1.51		0.43	0.53	7	27	6	45	18	8	15	60	135
1924																	
Submaintenance, 636.0 kgm.																	
Steer C																	
March 2–3	22	8	161	1.49			0.72	0.80	265	409	23	101	16	tr.	44	810	555
March 3	4	17	121	0.99	0.26	0.067	0.61	0.63	59		17	50	7	tr.	29	765	285
March 3–4	14	26	92	0.79	0.32	0.017	0.54	0.59	24	71	53	25	10	tr.	25	470	155
March 4	4	49	140	1.12	0.33	0.024	0.60	0.61	28	106	96	0	15	tr.	28	410	180
March 4–5	19	10	75	1.27	0.74	0.014	0.50	0.57	14	80	15	57	7	tr.	17	210	135

March 5	3	24															
March 5	7	2	92	1.46	0.93	0.046	0.54	0.69	17	53	54	7	8	tr.	13	120	130
March 5–6	22	46	67	1.61	0.94	0.026	0.58	0.79	12	37	14	9	4	tr.	9	80	120
March 6–7	14	56	61	1.63	1.06	0.032	0.56	0.79	13	31	19	2	1	tr.	10	65	120
March 7	10	11	62	1.62	1.01	0.060	0.57	0.65	9	29	14			2	7	65	120
March 7–8	14	41	62	1.61	1.08	0.046	0.56	0.60	9	29	11	23	9	3	7	70	115
March 8–9	23	12	65	1.61	1.10	0.041	0.54	0.57	8	26	9	13	15	tr.	8	85	110
March 9	9	57	64	1.70	1.12	0.060	0.62	0.63	9	29	24	3	15	tr.	10	90	125
March 9–10	14	1	76	1.68	1.19	0.049	0.61	0.60	11	29	29	14	8	5	10	125	120
March 10	8	42	69	1.60	1.11	0.060	0.58	0.56	12	26	40	12	3	2	10	140	120
March 10–11	15	53	68	1.56	1.05	0.053	0.57	0.56	11	27	50	14	11	5	9	150	115
March 11	8	19	59	1.52	1.06	0.059	0.59	0.58	11		57	10	10	2	10	135	110
March 11–12	18	26	58	1.43	1.08	0.029	0.58	0.58	9	24	53	14	11	4	12	140	110
March 12–13	20	39	52	1.44	1.01	0.053	0.56	0.60	9	24	48	16	9	3	10	115	115
March 13–14	24	46	52	1.39	0.92	0.069	0.56	0.60	13	35	71	0	27	2	15	115	125
March 14	7	5	62	1.48	0.88	0.096	0.64	0.65	18		84	57	6			170	
March 14–15	18	10	61	1.47	0.90	0.085	0.54	0.58	16	92	83	49	11	tr.	16	135	155
Submaintenance, 621.2 kgm.																	
Steer D																	
March 2–3	23	45	159	1.18					224	318	12	81	22	tr.	51	785	485
March 3	1	45	193	1.24	0.41	0.091	0.90	0.93	43				21	tr.		1045	
March 3–4	15	21	180	1.09	0.37	0.034	0.70	0.70	32	174	11	74	19	tr.	24	550	255
March 4	8	50	423	1.34	0.92	0.047	0.66	0.70	19	138	24	71	28	tr.	22	605	235
March 4–5	15	2	67	1.29	0.71	0.020	0.70	0.64	12	72	17	49	5	tr.	12	155	145
March 5	10	18	74	1.52	0.91	0.037	0.70	0.67	14	66	38	27	9	2	15	135	150
March 5–6	13	31	46	1.36	0.77	0.041	0.65	0.62	10	39	13	36	8	tr.	14	65	125
March 6	10	38	175	1.72	0.98	0.132	0.56	0.61	13	45	23	18	13	tr.	8	70	140
March 6–7	13	22	93	1.30	0.81	0.056	0.54	0.52	8	28	23	5	8	tr.	6	35	110
March 7	7	42	203	2.78	1.63	0.123	1.03	1.01	17	54	48	2	4	5	14	85	220
March 7–8	16	20	95	1.82	1.23	0.107	0.67	0.59	10	32	29	3	6	4	10	45	130

TABLE 2—*Continued*

FEED LEVEL, INITIAL BODY-WEIGHT, ANIMAL, AND DATE	DURATION OF PERIOD		VOLUME OF URINE	TOTAL NITROGEN	UREA NITROGEN	AMMONIA NITROGEN	PREFORMED CREATININE	TOTAL CREATININE	AMINO ACID NITROGEN	HIPPURIC ACID NITROGEN	INORGANIC SULPHATE (S)	ETHEREAL SULPHATE (S)	NEUTRAL SULPHUR (S)	ACETONE AND DIACETIC ACID	B-OXYBUTYRIC ACID	TOTAL FIXED BASES 0.1 N	ORGANIC ACIDS* 0.1 N
1924	*hrs.*	*mins.*	*cc.*	*grams*	*grams*	*grams*	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*cc.*	*cc.*
Submaintenance, 621.2 kgm.																	
Steer D																	
March 8	7	43	82	1.86	1.11	0.131	0.65	0.56	15	34	25	2	6	8	14	65	140
March 8–9	16	28	62	1.70	1.04	0.078	0.62	0.63	11	27	29	6	9	3	11	65	130
March 9	7	15	153	1.95	1.17	0.104	0.74	0.76	13	30	21	3	12	tr.	16	110	155
March 9–10	17	5	126	1.83	1.20	0.105	0.66	0.66	12	26	18	10	9	4	14	105	135
March 10	6	42	110	1.66	1.10	0.095	0.59	0.60	15	25	19	3		tr.	15	105	120
March 10–11	16	45	88	1.55	1.15	0.066	0.56	0.57	9	23	4	11	17	3	10	115	110
March 11	4	48	92	1.76	1.25	0.107	0.62	0.60	23					8	14	130	125
March 11–12	20	30	84	1.59	1.14	0.079	0.68	0.66	12	24	10	10	11	6	17	105	120
March 12	6	57	257	2.25	1.41	0.169	0.94	0.99	18	46	36	10	18	10	21	190	210
March 12–13	15	7	66	1.14	0.62	0.060	0.53	0.57	9	32	10	20	6	tr.	12	90	115
March 13	9	21	102	1.85	0.93	0.148	0.88	0.91	19	86	33	45	11	3	17	175	205
March 13–14	16	31	59	1.02	0.48	0.091	0.58	0.61	18	83	53	31	12	tr.	11	150	155
March 14	5	59	120	1.38	0.48	0.100	0.72	0.76	20	135	135	50	15	tr.	27	385	240
March 14–15	16	13	259	1.07	0.41	0.056	0.67	0.69	35	171	103	46	19	tr.	26	610	260
Submaintenance, 248.4 kgm.																	
Steer E																	
February 11–12	12±		58	0.62	0.11	0.236	0.07	0.13	93	35				0	3		240
February 12	12±		94	1.03			0.17	0.33	168	63				0	5		320
February 12	9	46	63	0.67	0.10	0.153	0.17	0.24	41	93				0	2		205
February 12–13	11	33	61	0.63	0.18	0.101	0.20	0.26	50	95				0	2		140
February 13–14	14	33	127	0.83	0.44	0.063	0.25	0.30	31	59				0	4		125

February 14	7	57	212	1.06	0.70	0.076	0.27	0.39	15	47				0	6	115
February 14	2	15	642	1.80	1.22	0.114	0.42	0.70	25	67				tr.	11	195
February 14–15	14	4	46	0.84	0.52	0.027	0.26	0.42	6	25				1	2	75
February 15	9	31	50	1.09	0.67	0.042	0.28	0.45	8					11	7	90
February 15	10	25	159	1.34	0.81	0.069	0.35	0.54	11					14	12	120
February 15–16	2	46	201	1.54	0.97	0.131	0.30	0.55	22					17	14	125
February 16	7	19	232	1.33			0.23	0.45	13	25				31	13	
February 16	6	14	215	1.37			0.25	0.48	35	12				23	16	145
February 16–17	11	37	137	1.32	0.67	0.145	0.27	0.47	13	26				31	20	110
February 17	10	2	85	1.12	0.60	0.145	0.24	0.40	19	20				31	19	95
February 17	6	30	76	1.69	0.84	0.129	0.54	0.82	14					13	18	205
February 17–18	16	2	38	0.75†	0.41†	0.127†	0.17†	0.28†	48†	33†				3†	4†	110†
February 18–19	14	4	69	0.80	0.17	0.136	0.20	0.26	64	137				1	6	205
February 19	12	51	79	0.69	0.18	0.055	0.24	0.31	64	124				tr.	9	215
February 19–20	11	26	87	0.69	0.13	0.077	0.28	0.30	58	176				0	7	245
February 20	13	24	96	0.63	0.07	0.056	0.27	0.30	51	146				0	10	240
Submaintenance, 277.0 kgm.																
Steer F																
February 11–12	12±		61	0.75	0.29	0.104	0.20	0.25	119	163				0	2	230
February 12	13±		65	0.89	0.36	0.095	0.23	0.30	72							
February 12–13	9	16	76	0.77	0.15	0.082	0.25	0.31	47	140				0	4	200
February 13	11	10														
February 13	1	33	83	0.95	0.41	0.123	0.34	0.41	66							
February 13	2	32	58	0.78	0.16	0.030	0.38	0.44	19							
February 13–14	22	15	47	0.82	0.51	0.029	0.29	0.34	21	48				0	1	95
February 14	2±		70	1.20			0.40	0.48								
February 14–15	20±		38	0.99	0.57	0.024	0.30	0.48	8	26				1	3	85
February 15	4	50	46	1.28	0.46	0.027	0.34	0.58	5							
February 15–16	18	21	46	1.40	0.91	0.054	0.37	0.58	11	22				6	3	100
February 16	6	24	45	1.36	0.86	0.066	0.32	0.50	5							

TABLE 2—*Concluded*

FEED LEVEL, INITIAL BODY-WEIGHT, ANIMAL, AND DATE	DURATION OF PERIOD		VOLUME OF URINE	TOTAL NITROGEN	UREA NITROGEN	AMMONIA NITROGEN	PREFORMED CREATININE	TOTAL CREATININE	AMINO ACID NITROGEN	HIPPURIC ACID NITROGEN	INORGANIC SULPHATE (S)	ETHEREAL SULPHATE (S)	NEUTRAL SULPHUR (S)	ACETONE AND DIACETIC ACID	B-OXYBUTYRIC ACID	TOTAL FIXED BASES 0.1 N	ORGANIC ACIDS* 0.1 N
1924	*hrs.*	*mins.*	*cc.*	*grams*	*grams*	*grams*	*grams*	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*cc.*	*cc.*
Submaintenance, 277.0 kgm.																	
Steer F																	
February 16–17	18	43	48	1.43	1.00	0.074	0.38	0.56	7	19				8	6		
February 17	6	31	45	1.27	0.88	0.081	0.30	0.43	6								
February 17–18	18	1	61	1.32	0.94	0.090	0.31	0.44	9	18				9	7		85
February 18	5	0	56	1.61	0.45	0.111	0.44	0.67	13								
February 18–19	20	5	46	1.15	0.58	0.076	0.29	0.45	11	37				3	5		110
February 19–20	11	4	74	1.05	0.56	0.090	0.30	0.33	23	114				tr.	4		175
February 20	12	36	54	0.66	0.23	0.059	0.20	0.22	20	106				0	6		175

* Not corrected for total creatinine.

† This urine was contaminated by a small amount of feces.

mostly between 1.61 and 1.43 for the remainder of the fast. With D in the same fast, there is somewhat more irregularity than with C. The volumes of urine per hour vary from 423 cc. on March 4 to 46 cc. on March 5 to 6. The nitrogen excretion is more irregular than with C. Steer F is much more regular in regard to the volume of urine and the nitrogen excretion and has a range of volume excretion that is narrow in rate per hour. In spite of the small and generally lower volume per hour in the latter part of the fast, the nitrogen per hour rises consistently and is much higher than in the earlier part of the fast.

From the results with these steers, it is apparent that there are variations among different animals (Forbes, Fries and Kriss, 1926). These results suggest that, where it is desired to obtain the periodic variations after the ingestion of food or from hour to hour with an ainmal, it would be wise to run preliminary experiments with different animals and then select those which show regularity in nitrogen excretion, for it is evident that C and F are regular in the voiding of the nitrogenous constituents, irrespective of the quantity of the urine, suggesting that at each voiding the bladder was emptied and that physiologically the animal functioned regularly.

Inorganic sulphate. The inorganic sulphate is relatively less than the ethereal sulphate in most of the fasts. In December, 1921, the amounts per hour were extremely low with both animals with the exception of the first day with C and the third day with D. In January, 1922, the initial value for C was low but not so low as most of the values in December, 1921. The inorganic sulphate rises during the first three days of the fast and then drops off. Both animals show a rapid decrease to less than one-fourth of this amount in the course of the 10-day fast. In a similar manner, the initial values in April, 1922, are low with a maximum on the fourth to fifth days. Then they fall off gradually during the rest of the fast. In November, 1922, there were maximum values on the third and fourth days and then a decrease to amounts per hour which are similar to those occurring in preceding fasts. The inorganic sulphate in November, 1923, is low and more regular. In the March, 1924, fast the values[4] are of about the same order of magnitude as for the other fasts, with a rise in the latter half of the fast with C and a rise in the middle of the 10-day fast with D followed by a fall in the latter half of the fast.

Ethereal sulphate. The ethereal sulphate values were in general higher at the beginning of the fasts than those for the inorganic sulphate, thus forming a greater proportion of the excretion of sulphur. They drop off

[4] The values are higher in many cases than those reported earlier (Proc. Nat. Acad. Sci., 1925, xi, 155) because repetition by the gravimetric method showed the presence of inorganic sulphate. In the earlier work the analyst omitted the titration (Fiske's method) with many of the urines, because of the absence of visible benzidine precipitate.

as the proportion of inorganic sulphate increases. The probable interpretation of this is that, as a result of the ingestion of food, there is putrefaction going on in the alimentary tract with the formation of materials which are coupled with sulphuric acid, and that, as the fast progresses, there is a lesser formation of these substances and an increase in the proportion of sulphuric acid in the form of inorganic sulphate. For the first few days, there is an increase in the proportion of inorganic sulphate and a decrease in the ethereal sulphate, and then the true effect of fasting comes on, so that there is a falling off in both materials, although the formation of ethereal sulphate does not cease.

The ethereal sulphate in March, 1924, was lower than in the other fasts. The amounts per hour were at the minimum on the fourth and fifth days for the two steers, but increased slightly in the latter half of the fast.

Neutral sulphur. There was less variation in the excretion of neutral sulphur than in the other two forms. In December, 1921, the values are highest on the first day. There was an immediate fall with a tendency to constancy for the rest of the fast. Likewise, in January, 1922, the amount is highest on the first day, drops off to a low value on the second day, and during the remainder of the fast reaches values which are comparable to those occurring in December, 1921. In April, 1922, low values were found for C at practically the beginning, slightly increased amounts occurred between the fifth and tenth days and then there was a falling off toward the end of the fast. The values in the June, 1922, fast were on the whole higher and with less range. In November, 1922, the elimination of neutral sulphur was lower for C than for D and with slightly more fluctuation. The values for the November, 1923, fast were materially higher than in any of the other fasts. There was a fall in the values for D but on the contrary there was an increase for C from the value on the first day. In the March, 1924, fast which occurred after a submaintenance ration, the neutral sulphur was very low at the start and remained low for the greater part of the fast.

Total sulphur. The total sulphur was highest at the beginning of the fast in practically all cases, and in general there was a fall which began almost immediately, the percentage drop in most of the fasts being large, 60 and 70 per cent. In March, 1924, the values were lower at the start than in any other fast except December, 1921. Steer C fell to a low value on the fourth day and then gradually increased to about the initial value by the end of the fast. With D, there was the usual marked fall.

Chlorides. In December, 1921, there was a pronounced fall in both cases during the first three days and then the values were at a constant level for the remaining days. In January, 1922, the fall from the first to the fourth day was even more marked. This was followed by a rise in the next three days, with a subsequent fall. In a similar manner with

steer C in April, 1922, the fall continued nine days and again there was the rise followed by a fall. With D, the rise in the latter part of the April fast was even more marked. Presumably, at first, the elimination of the chlorides diminished during the falling off of the store present in the body, and this took place until the point was reached when there was an elimination from the tissues and fluids of the body which contained chlorides. That is to say, the rise taking place after the initial fall was due to the true effect of fasting making itself felt upon the call for utilization of reserve material.

URINE AFTER PASTURE AND SUBMAINTENANCE. With the importance of preservation of urine and the necessity for adding hydrochloric acid, chlorine could not be determined, but with better preservation, more complete analysis of the urines, with special reference to the nitrogen partition could be made. Two types of fasting experiments were available; first, after a sojourn on pasture with steers C and D, and second, all four animals were studied after a preliminary long submaintenance period of feeding. The details of these studies are given in table 2, to which table subsequent discussion refers.

Urea plus ammonia-nitrogen. In November, 1923 (table 2) with C and D, fasting after pasture, the ammonia- and urea-nitrogen were determined together. The values gradually decreased from the beginning of the fast to the end and tend to parallel the changes in nitrogen elimination. The values are higher than would be the case for the fasts after submaintenance if the ammonia and urea were added together. A drop in the ammonia plus urea-nitrogen followed feeding with D. The urea-nitrogen and ammonia nitrogen were determined separately only in the fasts following submaintenance.

Urea-nitrogen. The amount was low at the beginning and gradually increased until March 6, 1924 it had reached the general level for the values throughout the remainder of the fast. With feed the urea-nitrogen fell off and with D more markedly than with C. Steers E and F show similar courses in urea excretion.

Ammonia-nitrogen. There is more regularity and a quicker approach to a general level with C than with D; also the values are in general lower for C. Feed increased the ammonia with C. The increase after food is less marked with D. Both E and F show a great irregularity in the ammonia, E more so than F, whose values are somewhat the lower. Food lowers the amount of nitrogen eliminated as ammonia.

Amino-acid nitrogen. In November, 1923, following pasture the amino-acid nitrogen falls from the first to the second day. It should be noted, however, that these November collections are for 24 hours, and that the other collections are all for shorter periods of time. Following submaintenance rations the drop is marked from the day preceding to the first

period with C and D in March, 1924, a level for both C and D being reached by the third or fourth day and for the remainder of the fast with C the amounts are constant. Food raises the value slightly with C, but not nearly to what it was before the fast began or even at the beginning of the fast. With D, the values are somewhat more irregular. Food does not immediately affect the amount eliminated by D although on the third day there is a tendency toward a rise in the amino-acid nitrogen. E shows a greater irregularity than C and D and although E and F weighed less than C and D, the amino-acid elimination is in general as high if not higher throughout the fast than with C and D. On the whole, F has a slightly lower elimination than E. Feeding raises the amino-acid nitrogen.

Hippuric-acid nitrogen. This determination involves solely the measurement of benzoic acid, but the nitrogen is calculated on the assumption that the benzoic acid represents the hippuric acid eliminated. With C and D in November, 1923, after pasture, the amount of hippuric-acid nitrogen falls from the first day until the third day. In March after submaintenance the values for both steers are higher on the one day before the fast than during the fast. With C, the resumption of food increased the hippuric acid to about three times the average during the fast. With D, the increase was even more marked by the third day.

The minimum values eliminated by E and F during the fast are similar to those of C and D in March, 1924. It is striking that animals which weighed so much less than C and D, eliminated nearly the same amount of hippuric-acid nitrogen during fasting. The change from the elimination previous to the fast to that during the fast is not so marked with E and F, and the fall to the minimum value is more gradual than with C and D. In both cases feed increases the hippuric-acid elimination, and in almost the same proportion as with D in March, 1924.

Preformed creatinine. Following pasture in November, 1923, the values were fairly constant for each animal. In March, 1924, the values with C were very regular throughout the fast, but with D the creatinine is both higher and more irregular.

The creatinine elimination for E and F was lower than for C and D, but these were younger and lighter weight animals. The values for E before the fast began were low. There was also a wider variation considering the amounts involved with E than with C and D. With F, the values are more regular and slightly higher than with E.

Total creatinine. In the fast of C and D, November 1923, there was a marked difference between the preformed and the total creatinine, which indicated, theoretically at least, a fairly constant and large elimination of creatine. The changes in the total creatinine were parallel to the changes in preformed creatinine. In March 1924 there is an indication with C that the difference between the two values obtains for only the first half

of the fast. From March 3 to 8 and 9 the difference gradually decreases and subsequently practically disappears even after food. In some cases the total creatinine is lower than the preformed creatinine.

With E and F, the differences between the preformed and the total creatinine were more marked than with C and D in the March 1924 fast, the difference continuing even after food, although it was not so marked with food as during fasting. It would appear that the smaller animals were more affected by fasting as shown by the elimination of creatine than the larger animals. The results with C and D would point to the elimination of creatine during the days with food. The disappearance of creatine during the fast is unusual as in general it has been found with fasting animals that creatine increases rather than decreases.

Its appearance after food is unusual and may not be due to metabolism but to decomposition of the urine in the bladder before it was voided. The urines were highly alkaline and it is probable that the retention of such an alkaline fluid at body temperature resulted in a change from creatinine to creatine. We have found that creatinine when added to an alkaline urine disappeared quickly so that in the urine as voided it is practically impossible to keep the creatinine for a long period of time. This supposition is of importance because it points to the possibility of the composition of urine being due, not solely to the elimination of the constituents, but to a chemical change which takes place before the urine is voided. Seemingly with steers the reaction of urine before it is voided and the length of time that it remained in the bladder must be considered in relation to its composition, in accordance with the findings of Pekelharing and Van Hoogenhuyze (1910) with rabbits.

Acetone and diacetic acid. With C and D, November, 1923, after pasture, there were but traces, and in March, 1924, for the great proportion of periods the maximum for either animal is less than 0.2 gram per 24 hours. During the food days there is a trace of acetone bodies eliminated and the effect of fasting is not to increase materially the elimination of these bodies. With E, the amounts are higher but the maximum is less than 0.75 gram per 24 hours, with F, in general showing less.

β-oxybutyric acid. The amounts of β-oxybutyric acid eliminated were low for a fasting animal. In no case is the amount for 24 hours over 1 gram, and in the majority of cases it is under 0.25 gram per 24 hours. It would appear that these animals always eliminated a trace, or small amounts, of β-oxybutyric acid, but that the effect of fasting is not an increased elimination or formation of acetone bodies and therefore there was practically a complete oxidation of fat. This is of interest because man, although subsisting on a mixed diet, begins to eliminate these bodies as soon as he fasts and the elimination increases as the fast progresses. In fact, these steers seemed better able to withstand a fast than does man.

Total fixed bases. After pasture the elimination of fixed bases at the beginning of the fast corresponded to over 1,200 cc. of tenth normal, but on the second day it had dropped to one-half and one-third of this amount and on the fourth day, with D, to one twenty-fifth of the amount eliminated on the first day. In March, 1924, the values for the day preceding the fast were not as high as on the initial day in November, 1923. In both cases the fixed bases dropped to a level and remained at about this level between March 5 and March 9. The elimination then rose to a higher level for the remainder of the fast. This fall at the beginning of the fast to a level for several days and then a rise indicates that at first there was an elimination of the surplus, or store, of fixed bases in the animal and that for a period of time during the fast this elimination was constant. Then the increase would point to an actual destruction of tissue or to a diminution of the body fluids containing fixed bases.

Organic acids. In November, 1923, there was a drop in the elimination of organic acids so that on the fourth day the values are about the same for both animals. In March, the fall from the day before the fast to the second or third day is as rapid as in November, 1923, and the values reached are about the same. From there on throughout the remainder of the fast, the amount of organic acid is practically constant with the exception of one day, March 7, with D. Feed raised the elimination of organic acid.

With E, the general average to which the organic acid dropped is nearly the same as with C and D in March, 1924. For F, the values are slightly lower than those with E. Feed raised the organic acid eliminated in both cases.

Proportion of organic acids as hippuric acid. In order to ascertain what causes the organic acid titration, an estimate has been made of the proportion of organic acids due to hippuric acid. This has been calculated on the basis that it will titrate completely as an organic acid. This is not strictly true, as according to Van Slyke and Palmer (1920) 90.2 per cent is dissociated at the hydrogen ion concentration to which organic acids are titrated, so that the values are theoretically about 10 per cent too high.

In November, 1923, the per cent of organic acids as hippuric acid ranged from 31 to 16 per cent with a gradual decline through four days of the fast. In March, 1924, with C, the first measurement on March 3 to 4 was 33 per cent. With the exception of the two periods which followed on March 4 to 5, the proportion gradually dropped until on March 6 to 7 it was 18 per cent and it remained between that figure and 15 per cent for the remainder of the fast. On the taking of food it rose to 43 per cent on the second day after the fast. With D, in the first two periods of the fast, it was 49 and 42 per cent. It remained between this figure and 14 per

cent for the rest of the fast. By the third period after the taking of food, on March 13, it was 30 per cent and on March 14 to 15 it was 47 per cent.

With E on February 12, it was 33 per cent. It rose to 49 per cent in the second period on February 12 to 13 and then varied between 34 and 5 per cent for the remainder of the fast. With F it was 50 per cent on February 12 to 13, and 15 per cent on February 17 to 18. On the taking of food, it rose to 46 and 44 per cent in the last two periods, February 19 to 20.

Hippuric acid, therefore, forms a greater proportion of the total organic acids during food than during fasting, and during fasting, with the larger animals, it may drop to a constant figure, after about three days.

Proportion of organic acids as creatinine. In November, 1923, the organic acids as creatinine varied for C and D from 15 to 38 per cent.

In March, 1924, the creatinine for C the first day of the fast was 19 per cent of the organic acids and then rose in the latter half of that day to 31 per cent. In the latter half of March 5 to 6, the third day of the fast, it was 44 per cent, and between this and March 13 to 14, the first day after the fast, it varied from 48 to 40 per cent. With D, the creatinine was 24 per cent in the latter half of March 3 to 4, the first day of the fast, and 25 per cent in the first period of March 4 to 5. Both animals eliminated between 40 and 45 per cent of the total organic acids as creatinine during the last 7 days of the fast.

With E, in the first period of the fast on February 12, it was 7 per cent. By February 14 to 15, it was 31 per cent and varied between 27 and 15 per cent for the remainder of the fast. With E and F, which are somewhat younger and smaller animals, the proportion of organic acid eliminated as creatinine is lower than with C and D.

The sum of creatinine and hippuric acid. The combined proportions were between 58 and 66 per cent for the greater part of the fasts with C and D, so that the amount of organic acids to account for is about 35 per cent. Food does not affect materially the sum of the proportions for creatinine and hippuric acid.

With E and F, the sums of the proportions are in general much lower. At the beginning of the fast of E the value for the period is 40 per cent. The range is between 20 and 61 per cent. The few scattered results with F, during food and fasting, varied between 47 and 61 per cent.

Organic acids per kilogram per 24 hours. Van Slyke and Palmer (1920) reported the organic acids per kilogram per 24 hours excreted in the urine of normal young men ranging from 5.7 to 9.8, with an average of 8.2 cc. These values were uncorrected for creatinine as also are ours. A similar calculation for these animals shows that in the fast after pasture it varied between 10.0 and 5.5 cc. With C in March, 1924, on the day before the fast it was 20.9 cc.; with D it was 18.7, but it immediately fell to 7.0 with C and 9.8 with D on the first day of the fast. Beginning with March 5 to 6,

the values for C were regular, i.e., 4.4 to 4.8 cc.; with D the values were more irregular, varying after the first day from 4.6 to 6.8.

With E and F, the organic acids per kilogram per 24 hours were 27.3 cc. with E on the day before the fast and 20.2 with F. The lowest value for E during the fast was 8.2 on the third day, the amount varying between 16.3 and 8.2. With F it was 17.3 on the first day of the fast and then dropped to 8.7 on the second day and varied between 9.4 and 7.7 for the remainder of the fast. The values during feeding with these animals are, therefore, somewhat higher than was found for young men. During fasting with the mature animals, the amount falls to a fairly constant figure and remains constant after the fourth to fifth day of the fast. There is more variation in the excretion per kilogram of body weight with the younger steers, E and F, than with the older animals, C and D.

NITROGEN PER KILOGRAM PER 24 HOURS. It is evident that there may be different levels of nitrogen per kilogram according to the previous conditions of the animal. For example, the lowest average nitrogen per kilogram per 24 hours with C is following submaintenance in the March, 1924, fast, in which the highest value is but 0.067. The next lowest is the fast in April, 1922, which in general runs similar to the one in January, 1922, when the final level of about 0.075 is reached. The other four fasts are more or less comparable among themselves in the general level, 0.115 to 0.095, which is reached by the nitrogen per kilogram figures. The weights of the steer, however, were different in these other four fasts. The lowest weight was in December, 1921, 589 to 538 kgm., and the highest weight in November, 1923, 724 to 656 kgm. In spite of this, however, the nitrogen per kilogram figures were at approximately the same level, although there is as much as 120 kilograms difference in weight. The values with D were of about the same order of magnitude as those of C.

Steers E and F. Steers E and F excreted, respectively, on the first day of the fast, 0.062 and 0.069 gram, and the excretion rose to 0.13 to 0.14 during the fast. The taking of food by E on the next three days lowered the nitrogen per kilogram per 24 hours to 0.064, and with F to 0.078 gram. With these younger animals presumably the reserves were not as great as with C and D and consequently, the demand for energy supply was met by an increasing utilization of the protein in their bodies. This is corroborated by the fact that the taking of food gradually decreased the nitrogen per kgm. per 24 hours. Baer's (1906) results with fasting goats give 0.28 to 0.29 gram per kilogram per 24 hours, and Palladin's (1924a) values for sheep were 0.071 on the ninth day and 0.138 with a second animal at the end of a 16 day fast. F. G. Benedict (1915) found as the lowest value in a 31-day fast with man 0.146 gram, so that in general the fasting steers came to low values as compared with other ruminants and man.

CREATININE COEFFICIENT. The coefficients for C in November, 1923,

varied from 17 to 22, with a gradual increase throughout the fast. For D, the value on the first day was 18 and on the three remaining days of the fast 26, 27 and 24.

In March, 1924, the creatinine coefficient for C on the first day was 21. The lowest value was 20 on the next day, but the coefficients for the remainder of the fast ranged between 24 and 21. The two days of food after the fast gave similar values. The coefficient for D was slightly higher in general than for C.

For E on February 11 to 12 it was 18, and then for the remaining four days it varied between 27 and 29, being slightly smaller after feed. F gave similar values.

The creatinine coefficient may be taken as an indication of reserve material, that is to say, as to whether the animal is fat or lean, because the fatter the animal, the lower will be the creatinine coefficient, as was pointed out by Folin (1905b, p. 85). There is not so great a difference between the fasts of November, 1923, and of March, 1924, with C and D in the creatinine coefficients, as difference in weight (700 kgm. versus 635 kgm.) would lead one to expect. The coefficients are of about the same size because the elimination of creatinine is slightly higher in the November fast. With animals E (248 kgm.) and F (277 kgm.) the creatinine coefficients were high. This may be due to more active protoplasmic tissue (Folin, 1905b), from the fact that they were younger animals, or it may be due to a lower reserve. Probably both factors played a rôle.

With the fasting man studied at the Nutrition Laboratory (Benedict, 1915, p. 262), the creatinine coefficient varied from 21.5 on the first day to 25.3 on the fourth day of the fast. The values then gradually decreased and the minimum value was 18.1 on the last or thirty-first day. The coefficients were slightly lower for man than those in general found for the fasting steers. The creatinine coefficients of adult sheep, studied by Palladin (1924b), varied during fasting from 15.5 to 20.0.

Percentage distribution of nitrogen. *Urea. Steer C*, March, 1924. The percentage of nitrogen in the form of urea rose rapidly in the first period of the fast until March 4 to 5. From then on it gradually rose until it reached 75.5 per cent in the next to the last period on March 11–12, being for the most part over 60 per cent after the second day of the fast. Feeding on March 13 resulted in lowering the value about 10 per cent in the next two days as compared with the last period of the fast. *Steer D*, March, 1924. The percentage of total nitrogen as urea followed about the same course and rose to about the same maximum during the fast as with C. Food produced a much smaller elimination of urea than did fasting, the percentage falling to 34.7 in 2 days after the taking of food. *Steers E and F*, February, 1924. The nitrogen of urea on the day previous to the fast of E was 18.2 per cent of the total nitrogen and in the first

period of the fast on February 12, 15.6 per cent. Inside of two days it rose to 68.1 per cent, which is similar to the maximum value found with C and D. It remained between this figure and 50.4 per cent for the remainder of the fast. The taking of food by E, which began on February 17, caused a drop in the percentage of the total nitrogen. With F, the proportion of the total nitrogen as urea was somewhat higher on the day previous to the fast than with E. It rose rapidly during fasting until it was 62.6 per cent on the second to the third days. For some unaccountable reason the urea-nitrogen on February 18, immediately following the fast, was only 28.2[5] per cent, but it rose again to 52.7 on February 19 to 20 though falling to 35.8 in the last period with food.

Ammonia. Steer C, March, 1924. The values are, for the most part, under 7 per cent and indicate clearly that there was no call for extra ammonia to neutralize any acid formation which was excreted. The figures are regular for most of the fast. *Steer D*, March, 1924. The values for the percentage of ammonia-nitrogen with D are slightly higher than with C. In general, however, they are under 7 and over 3 per cent. The taking of food raised the value. *Steers E and F.* The ammonia on the day before the fast of E was 38.1 per cent of the total nitrogen, a high value which would indicate a decomposition of the urine. Even on February 12, it was 22.7 per cent but in the third period it was 7.6 and dropped to a minimum of 3.2 on the third day. At the end of the fast, on February 17, the percentage rose to 12.9. The taking of food on February 17 resulted in variable values between 7.6 and 17.0 per cent. With F, the values preceding the fast were 13.9 and 10.7 per cent. The percentage fell during the fast from 10.6 at the beginning to a low value of 2.1 on February 15, and then rose to 6.8 at the end, on February 17–18. Food did not materially change the percentage.

Urea nitrogen plus ammonia nitrogen. Steers C and D, November, 1923. The urea and ammonia were not determined separately in the November, 1923, fasts of C and D but together on each day, and were between 65.7 and 82.7 per cent of the total nitrogen during the 4 and 5 days of the fast. These values are materially higher than the sums of the percentages for these two nitrogenous substances found in the early days of fasting with C and D in March, 1924, and with E and F in February, 1924.

Amino-acids. With steers C and D the nitrogen of amino acids ranged between 0.3 and 2.0 per cent of the total nitrogen in the fast in November, 1923, and the highest value for both steers occurred on the first day. In March, 1924, the amino-acid nitrogen excreted by C was 17.8 per cent of the total nitrogen on the day preceding the fast, and fell off rapidly with fasting until March 5–6, after which it remained between 0.8 and 0.5

[5] One might assume that the low value was due to decomposition, but the percentages of the other constituents do not indicate this.

per cent. Feeding on March 13 raised the elimination slightly. With D the findings were essentially similar. In the case of E the amino-acids constituted 15.0 and 16.3 per cent of the total nitrogen in the two periods of the day before the fast, and with F, 15.9 and 8.1 per cent. With both of these steers the percentage dropped to a low value in three days of fasting. The taking of food raised the values, a maximum of 9.3 per cent being noted with E in six successive periods on three days and of 3.0 per cent with F in two days after the resumption of food.

Hippuric acid. The proportion of total nitrogen eliminated in the form of hippuric acid varied between 3.7 and 1.3 per cent during the 4- and 5-day fasts of C and D in November, 1923, and decreased as the fast progressed. In the March, 1924, fast of C it was 27.5 per cent on the day preceding the fast and by March 5 to 6 it had fallen to 2.3 per cent and was under 2 per cent for the remainder of the fast. When C was fed on March 13 there was a slight rise, and on March 14 to 15 the value was 6.2 per cent. Thus, during the food days the proportion of hippuric-acid nitrogen was comparatively high, but as the fast progressed it rapidly fell off until it reached a practically constant value. The decrease in the hippuric-acid nitrogen excreted by D in the March, 1924, fast was about the same as noted with C.

In February, 1924, the hippuric-acid nitrogen excreted by E on the day preceding the fast was only about 6 per cent of the total nitrogen. In the two periods on the first day of the fast it was 13.8 and 15.2 per cent, but it dropped during the fast to 0.9 per cent on February 16. The taking of food after the fast raised the value to a level more nearly like that observed with C and D during feeding.

Preformed creatinine. In the 4- and 5-day fasts of C and D in November, 1923, the percentage of the total nitrogen as preformed creatinine varied between 5.2 and 9.3, increasing with each steer as the fast progressed. In the March, 1924, fast of C, one of the initial values was as high as 25 per cent, but by March 4 to 5 it had dropped to 14.7 per cent, and remained between 12.6 and 15 per cent for the remainder of the fast. These values are similar to those found by Folin (1905b) with man on a starch-paste cream diet. In the March fast of D the values are much like those noted with C. The preformed creatinine nitrogen constituted with E in February, 1924, a lower percentage of the total nitrogen, in general, than it did with C and D in March, 1924. When E was fed after the fast, the values increased somewhat, the maximum value being 16.2 per cent on February 20. Similarly, with F on the day before the fast, the proportion was about 10 per cent and rose to 18.2 per cent on February 13, the second day of the fast. The lowest percentage during the fast was 8.7. After the fast the maximum value was 11.1 per cent. The low values observed during the fasts of these younger animals may be accounted for in two ways.

There may have been a lower elimination of creatinine, but, as previously shown, the creatinine coefficients of E and F were within the range of normal values, and, in fact, were slightly higher than those for C and D. It is therefore probable that the low percentages are not due to a low excretion of creatinine. On the other hand, there may have been a relatively high excretion of nitrogen. That this is so is indicated by the fact that with E and F the nitrogen per kilogram of body-weight per 24 hours, instead of decreasing as it usually did with C and D, increased throughout the fast. The percentage of nitrogen as creatinine may be taken as an indication of the nitrogen level (nitrogen per kilogram of body-weight) in the different fasts. If this percentage is used as an index, the nitrogen level of C and D is highest in November, 1923, following pasture, and lowest in March, 1924, after the submaintenance ration, whereas in February, 1924, with the smaller animals, E and F, the level is higher than in March, 1924, with C and D. In all the fasts the percentage of nitrogen as creatinine is higher than that found in the urine of the Nutrition Laboratory fasting man (Benedict 1915, p. 256), for in the majority of his urines the creatinine constituted under 4 per cent of the total nitrogen.

Total creatinine. If the nitrogen in total creatinine is greater than that in preformed creatinine, the difference is ascribed to creatine. In some cases, particularly in March, 1924, with C and D, the total creatinine was even less than the preformed creatinine. This might be due to the difference in the method of determining the preformed and the total creatinine, as has been shown by Greenwald (1913), for any aceto-acetic acid present in the urine would, by the method of analysis, be lost from the urine sample by heating in the determination of the total creatinine, but would not be lost in the determination of the preformed creatinine because there would be no heating of the sample. The acetone bodies in these urines are so low, however, that it is questionable whether they could play any rôle in affecting the determination of preformed creatinine. There seems to be no relation between the amount of acetone bodies in the urine and the absence of creatine. The values for total creatinine in the fast of E and F indicate clearly the presence of creatine, as they are greater than the values for preformed creatinine all through the fast. Yet it is in this fast that the highest values for acetone bodies are found, viz., steer E, February 16 to 17 and February 17. Similarly, in the latter part of the fast of November, 1923, a marked difference was noted between the preformed and total creatinine, although acetone values were also found which were higher than at the beginning of the fast.

In November, 1923, the greatest percentage of nitrogen due to creatine (total minus preformed creatinine) was 4.7 on November 7 to 8 with C. On the other days it was 4 per cent or under. In March, 1924, with C and D, it was only at the beginning or in the early portion of the fast and

when food was eaten that there was any real difference between the percentages of total and preformed creatinine-nitrogen. With E and F, the percentages were higher for total creatinine than for preformed creatinine throughout the fast. Feeding does not result in a marked difference between the percentages, although the values for the total creatinine are always higher than for the preformed creatinine.

The fact that with E and F the values for total creatinine were higher than for preformed creatinine indicates that there was an excretion of creatine throughout the fast which was contrary to the findings with C and D. One explanation of this might be that the reserve store of these two animals, E and F, was not sufficient to supply an adequate amount of energy from carbohydrate and fat so that the animals gradually had to call upon their store of protein, and this disintegration of tissue resulted in a liberation of creatine. Such a result was pointed out by Benedict (1907) in the earlier studies on fasting man. The difference between the two pairs of animals is interesting in view of the results of McCollum and Steenbock (1912) who found that growing pigs did not excrete creatine during fasting. They ascribed the absence of creatine in the urine to the ability of the animals to utilize their fat efficiently without the necessity of drawing upon organized protein material.

The difference with regard to creatine in the fasts of C and D and E and F does not confirm the general finding that creatine is excreted because of the withdrawal of carbohydrate. There is, however, even with E and F an unexplainable condition in this connection because there is very little excretion of β-oxybutyric acid and acetone bodies, a fact which would indicate that the fat was completely metabolized as with a normal animal. Probably the store of fat in E and F was not sufficient to form a proportion of the total metabolism which would keep down the inroad upon the protein store. Presumably the proportions of the total metabolism due to protein and fat are in part, at least, determined by the original composition of the animal.

Rest-nitrogen. The percentage of undetermined nitrogen is of value in indicating the probable correctness of the amounts of nitrogen in individual constituents, because if the sum of the different percentages other than undetermined nitrogen is considerably above or below 100, there must be an error in one or several determinations. The percentage of rest-nitrogen is also of value as an indication of the presence of significant amounts of undetermined substance of nitrogenous character.

The undetermined nitrogen with C and D in November, 1923, varies between 21.4 and 4.3 per cent. In March, 1924, it was for the most part under 20 per cent. The percentage tended to decrease as this fast went on, so that the elimination of unknown material containing nitrogen was gradually decreasing. On the resumption of food there is a slight

increase so that, whatever the unknown material is, some time is required before it appears in the urine after feeding.

The values for the undetermined nitrogen with E and F are, in general, similar to those for C and D in March, 1924. On the food days following the fast the values are somewhat higher than during the fast.

DISTRIBUTION OF SULPHUR AND NITROGEN TO SULPHUR RATIO. *Inorganic sulphates.* In most of the fasts, other than those of December 1921 and November 1923, the sulphur of inorganic sulphates is a low percentage of the total sulphur at the start of the fast and rises sharply by the third or fourth day to a level where it is the predominating form in which sulphur appears in the urine. After the third to fourth days there is a tendency to constancy. The values in March 1924 are more irregular than in the other fasts in which the maximum is reached in three to four days. In December 1921 and November 1923 the values are low for the most part and remain so throughout the fasts. In June 1922, the rise is sharp only in the first three days with steer D and in general the values are between the two groups discussed previously. The low values at the beginning of the fasts are generally under 25 per cent, and the high level to which the inorganic sulphate rises is between 50 and 80 per cent.

Ethereal sulphates. In contrast to the inorganic sulphates, the percentage of total sulphur as etheral sulphates was relatively high at the beginning of the fasts and tended to decrease as the fast progressed. In December 1921 this tendency was less marked. In January 1922 the percentages with C are in general higher than with D. With C there is a fall in the first six days and then a rise for two days, succeeded by a fall. With D, from the second day to the seventh day there is also a marked drop, after which the percentages are generally under 30 per cent for the remainder of the fast. In June 1922, the initial level was not so high as in the preceding fast and the fall was not so sharp. In November 1922, the proportion fell for both animals to a minimum on the third or fourth day and then rose to about 30 per cent in the remainder of the 9- and 8-day fasts. The percentage on the day preceding the fast of March 1924 was over 70 with both steers and was about the same in the first period measured during the fast. The values fell in the first two or three days to under 40 per cent and continued irregular for the remainder of the fast. With C, they were for the most part under 30 per cent after the third day; with D, they were mostly under 10 per cent from the fourth to the seventh day.

In general, then, at the beginning of the fast, the ethereal sulphates are higher in percentage of the total sulphur and may remain near 80 per cent or diminish to a relatively low percentage, depending apparently upon the preceding ration and whether any food residue remains in the alimentary tract. The cause of the high initial percentages is probably the putrefaction which takes place in the alimentary tract of the ruminant.

Neutral sulphur. In the investigations of Folin (1905b) it was found that the neutral sulphur was relatively a constant quantity, resembling the creatinine in its elimination, and that its percentage of the total sulphur depended upon the absolute amount of total sulphur excretion. The percentage as neutral sulphur in the majority of the steers' urines approximated a general value in each fast, but the magnitude of the percentage varied with the particular fast. In December 1921 and November 1923 the percentages were mainly higher at the beginning than in the other fasts, that is, nearly 50 per cent. The percentages in November 1923 were, in general, relatively high. In January 1922 with C, the values were for the greater part under 20, with a minimum of about 10 per cent, and with D between 15 and 30 per cent. The range in the June 1922 fast was not wide, the values varying from 15 to 30 per cent. In November 1922, the percentages were usually under 25. In the March (submaintenance) 1924 fast, there was greater irregularity on the whole than in any of the other fasts, the values with the two steers ranging between 4 and 53 per cent. In general, therefore, it is difficult to predict what the percentage of total sulphur as neutral sulphur will be in fasting steers' urines. It may be relatively high, it may correspond to the percentages found in man, or it may be low and also irregular. The values on days preceding the fasts were comparatively low, and on days following the fasts were materially lower than they were during the fasts.

Nitrogen to sulphur ratio. In December 1921, the values at the beginning are somewhat like the nitrogen-sulphur ratios found in human urines, but they rapidly rise till they are about 60:1 on the fourth and fifth days and are over 40:1 for the remainder of the fast. In contrast to this fast, January, April, June and November, 1922 gave ratios which are for the most part within narrow limits. The minimum in these four fasts was 17:1 and the maximum was 31:1. With D in April 1922, there was a tendency toward constancy in this ratio, with values ranging between 20:1 and 28:1 for the entire fast and between 23:1 and 26:1 for the last ten days of the fast. In March 1924, the initial values, both on the day before the fast began and at the beginning of the fast, were low. These were followed by a rise to very high ratios on about the fourth to fifth days. With C the values descend after the fourth day to about 20:1 and the effect of food ingestion after the fast is to diminish the value still further to about the same ratio as that preceding the fast. With D, the ratios remain high after the fourth day to the end of the fast, but on the days following the fast the drop is very marked, ratios of about 7:1 being reached in the last two periods. In general, then, the nitrogen-sulphur ratios with steers which have been on a full ration, in which grain is included, are constant during fasting, but when the preceding ration has been a submaintenance one, the ratio may rise to unusually high values.

GENERAL DISCUSSION. The study of the composition of the urine of fasting steers affords an opportunity for noting the changes which occur in an animal when it changes from an herbivorous to a carnivorous animal. At the beginning of the fast the animal has in the alimentary tract a considerable amount of unabsorbed and undigested material and with an animal as large as a steer this material remains in the alimentary tract for several days. During fasting the animal changes from a condition in which it exists on vegetable food to one in which, in all probability, its sustenance consists of its own tissues.

The urines of the steers at first are highly alkaline. Gradually the alkalinity disappears and after four or five days the urines react acid. During this period they have changed materially. The most marked change in the character of the urine is in the nitrogen distribution. At first, the urines are composed of materials which come from the food. Among these is hippuric acid which occurs in large quantities but, as the effect of previous food disappears, the hippuric acid gradually diminishes. On the contrary, urea, which one finds as a high percentage in the urine of humans and of carnivora, is in steer's urine a much smaller quantity. When food is withdrawn, the urea is increased so that the first change which takes place is a reversal of the proportions between these two constituents, that is, a lowering of the nitrogen due to hippuric acid and a gradual increase of the nitrogen due to urea.

In the fasts of C and D in which these constituents were studied the urea nitrogen figures finally approach a percentage of the total nitrogen which is like the urea percentage in the urines of men on a low nitrogen level. This level is also indicated by the relatively high percentage of nitrogen due to creatinine. Likewise, the amino acids are relatively high at the start and then fall to a low figure which plays but little rôle in the nitrogen distribution.

Nitrogen economy of steers. The determination of hippuric acid was really a determination of the benzoic acid rather than hippuric acid itself, and consequently we do not know whether there may not have been a hydrolysis of the hippuric acid in the bladder so that amino acid was set free and consequently measured in the amino-acid determination. If this amino acid was not free but was combined with benzoic acid, we have then, besides the free amino acids, considerable amounts of nitrogen eliminated in the form of combined amino acid and benzoic acid. When we take into consideration the low digestibility and absorption of many of the foodstuffs, particularly the coarse fodders which are used by ruminants, and then add to that the elimination of amino acids, either free or combined with benzoic acid in the form of hippuric acid, we see that the utilization of nitrogen by the steer may be extremely low and consequently as a source of obtaining protein economically from the nitrogen cycle,

this animal may be ineffective. When the formation of protein is the main object of feeding, it seems from the results of these urine analyses that it is important to know what portion of the nitrogen escapes into the urine in amino-acid form and as hippuric acid. It would be important, therefore, to determine which of various types of ration, that is, rations which are mainly hay and grasses or rations combined with various grains would result in a more economical use of the protein ingested. Studies are needed in which the hippuric acid as such is determined, as well as the benzoic acid, in order to ascertain whether amino acid from hydrolysis of the hippuric acid is eliminated in the combined or free form, together with the determination of amino acids as such. That this feature of the hippuric acid metabolism is being recognized is shown by the appearance recently of a number of papers in which methods are being used for the determination of hippuric acid and benzoic acid separately. These animals were on an extremely low nitrogen level and consequently we do not know whether, with a high nitrogen level of feeding, more would be eliminated as urea and relatively less as amino acids and hippuric acid or whether the reverse would be true. It can be seen from the course of the percentage distribution that, as the animal tends to live on itself more and more, the composition of the urine and the distribution of nitrogen become more like those of the ordinary carnivorous animal, or man, and it would seem that the nearer the ration of the animal was to its natural food intake, the greater proportionately was the loss of nitrogen in forms which had not undergone metabolism or become an integral part of the body.

Inorganic phosphates. In a few urines of April 1922 and November 1923, the inorganic phosphates were determined. The amounts found were very low, 0.7 to 5.7 mgm. per hour so that the excretion of phosphates in the urine of fasting steers is insignificant.

Phenols. The data for the phenols have already been given (Carpenter, 1925). In general they drop rapidly from the first to the third day and remain at a level for the rest of the fast and rise slightly after feeding in March 1924. The average distribution is 40 to 48 per cent for the free phenols and 52 to 60 per cent for the conjugated phenols. The effect of fasting is to diminish the production of phenols suggesting that putrefaction in the alimentary tract diminishes, but the continued slight excretion of these bodies would indicate that unabsorbed material remained for a long period of time in the tract.

Acidosis. Another feature of these urines is the low ammonia content. One would expect that, in the change from a ration entirely vegetable to one of flesh, the animals would develop acidosis, in other words, that they would have a low resistance to fasting and would show by the composition of the urine as great (if not a greater) an ill-effect as man and other animals which develop acidosis with an increase of ammonia in the urine. This

absence of acidosis is entirely confirmed by the extraordinarily small amounts of acetone bodies. In this regard steers differ entirely from man, who develops ketonuria fairly promptly. The younger animals, E and F, showed the same resistance to fasting so far as the development of acidosis and ketonuria is concerned. The low ammonia nitrogen percentage (under 10 per cent) of the urine during fasting brings the steer into the class of the dog (Underhill and Kleiner, 1908), rabbit (Mendel and Rose, 1911), cat (Fiske, 1923) and pig (Folin and Denis, 1915). In contrast to the results with these animals and steers are the findings by F. G. Benedict (1915, p. 256) in the urine of a fasting man.

The absence of development of acidosis and ketonuria was not due with these animals to the fact that they had a high proportion of carbohydrate in their metabolism. As will be shown in a forthcoming monograph by Benedict and Ritzman (1927), the respiratory quotient of these steers approached fasting quotients well below the ratio which Shaffer (1921a) has calculated for the development of ketonuria. The keto-antiketogenic ratio is being applied clinically for men but, when it is attempted to apply it for other animals, the result is not what one would expect if this were a universal biological phenomenon, that is, the development of ketone bodies when the proportion of carbohydrate to fat is low. It may fairly be questioned as to whether the cause of the development of ketosis on the part of man is due to a low proportion of carbohydrate in the metabolism or is due to the character of the material which is drawn upon when carbohydrate is diminished in the diet. That humans vary with respect to the onset of acidosis has been shown by Folin and Denis (1915) in an investigation in which they subjected two very fat women to repeated fasts. Means (1915) studied the respiratory exchange of one of them and found in each succeeding fast a higher respiratory quotient on the first day of the fast, and Shaffer (1921b) has shown that the appearance of of acetone bodies corresponds in the second and third fasts with this subject to expectancy from the respiratory quotients. This investigation (Folin and Denis), therefore, indicated that it is possible for a human being to gradually become accustomed to fasting. Is it an adaptation to fasting or is it due to the disappearance of material, the character of which leads to acidosis? Why man should differ as he does from the other animals, which in their metabolism show other characteristics which are similar to those found in man (that is, the proportions of protein, carbohydrate, and fat burned in the body), is strange and is a problem which demands attention from a standpoint other than the matter of the pure relationship of carbohydrate and fat.

A characteristic of steers C and D in the March 1924 fast, brought out by the analyses of the urines, was that they were on a very low nitrogen plane when they began to fast and yet, in spite of fasting, they did not

draw to any greater extent upon their protein but drew their energy mainly from fat. In connection with the low nitrogen output of these animals and of the two fasting women and their ability to use fat, it should be recalled that the treatment of diabetes by means of high fat and low protein has been advocated by Maignon (1908), Newburgh and Marsh (1920) and Petrén (1924). The question then is whether the ability to use fat is due to the extraordinarily low nitrogen level upon which the animal has been previously subsisting or to a natural ability to utilize fat in conditions of carbohydrate withdrawal. In other words, does a low nitrogen level predispose to a resistance to fasting?

Cattle are not subjected to great privations so far as food is concerned, at least under usual domestic conditions. In other parts of the world, however, ruminants do have to undergo periods of undernutrition. In South Africa, during the dry season, the vegetation becomes very sparse, it is difficult for the cattle to obtain an adequate ration, and they grow thin and waste in flesh considerably. Also, presumably, reindeer in the North during the greater part of the year must be obliged to exist on rather scant rations, so that it may be that ruminants in general possess the ability to subsist at a low nutritive level.

SUMMARY

In 5 fasts of 5 to 14 days with two adult steers (circa 600 kgm.) the volume, the total nitrogen, the inorganic, ethereal, and neutral sulphurs, and the chlorides were determined. In still another 5-day fast after pasture and a 10-day fast after submaintenance feeding, determinations were made of urea, ammonia, amino-acid nitrogen, hippuric acid, preformed and total creatinine, acetone bodies (acetone and aceto-acetic acid), β-oxybutyric acid, total fixed bases, and titratable organic acids as well as the constituents mentioned above (chlorides excepted). In a 5-day and 6-day fast, respectively, of two younger steers (circa 250 kgm.), all the constituents were determined except chlorides, sulphurs, and total fixed bases. The urines of the 5- and 10-day fasts were preserved by excess of hydrochloric acid which prevented changes in composition particularly in the distribution of the nitrogenous constituents.

The minimum nitrogen excretion of the adult steers was from 1.6 to 1.7 grams per hour, and between 0.064 and 0.075 gram per kilogram of bodyweight per 24 hours. The general trend in the rate of elimination of urinary constituents was a falling off, depending upon the previous ration, with the exception of the rise in inorganic sulphates and the marked increase in urea, determined only in fasts after submaintenance feeding. The greatest lowering took place in the first 4 to 6 days. The rise in the chlorides and total fixed bases after the fall in the excretion is evidence

of the actual destruction of tissue or a diminution of the body fluids with the progress of the fast. An exception to the general trend in the total nitrogen excretion was the increase during the fasts after submaintenance rations with both the adult and the younger steers.

The creatinine coefficient (milligrams of creatinine per kilogram of body-weight per 24 hours) was similar to those with other mammals. There was no effect upon this value due to fasting.

In a short fast with the adult animals creatine was present, whereas in the 9-day and 10-day fasts after submaintenance it was present only in the early portion of the fast and disappeared as the fast progressed. With the younger animals the creatine increased.

The inorganic phosphate was determined in a few urines and it was found that the excretion was very low.

A calculation of the nitrogen partition showed a lowering of the amino-acid and hippuric-acid nitrogen percentages during fasting, an increasing urea-nitrogen percentage, and an approach in general to a nitrogen distribution, so far as ammonia, urea, and creatinine are concerned, similar to that found with men on a low nitrogen level. Striking results were the poor economy of the nitrogen metabolism of steers as indicated by the relatively high proportions of the total nitrogen as hippuric-acid nitrogen and amino-acid nitrogen at the beginning of the fasts and on the resumption of food, and the absence of acidosis as shown by the low percentage of ammonia nitrogen and the practically negligible elimination of ketone bodies.

The distribution of sulphur showed a low inorganic and correspondingly high ethereal sulphate percentage at the start, with a reversal in the relative proportions as the fast progressed. The neutral sulphur percentages showed rather wide variations. The nitrogen to sulphur ratio was for the most part under 25:1, but in two fasts it rose to an unusually high ratio.

The fasting base-line, or the disappearance of the effect of previous food, is reached by the fourth to the sixth day, as indicated by the constancy reached in this interval by the chlorides, phenols, fixed bases, organic acids and nitrogenous constituents.

(Complete publication of the results of the investigation upon the metabolism of fasting steers is given in Publication 377, Carnegie Institution of Washington, by Professors Francis G. Benedict and Ernest G. Ritzman, to whom I am indebted for securing the material used in the study reported here. The analyses were made by Miss Dorothy M. Tibbetts and Messrs. Y. Habeshian, Philip P. Saponaro and Edward S. Mills.)

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I. THE EFFECT OF THE PARATHYROID HORMONE ON GASTRIC SECRETION

II. The Calcium Content of Gastric Juice

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An extract of the parathyroid glands which may be used to prevent certain types of tetany, and which elevates the blood calcium to a remarkable degree, was first described by Collip (1925). In some studies of the effects of overdosage on the dog with the extract he noted that, after injections of large amounts of the material, now called "Parathormone," the animal may begin to vomit bile and later blood. The animals dying from the effects of overdosage showed at autopsy a dark and hemorrhagic mucosa of the stomach as one of the most singular findings (Collip, 1926).

Matthews and Austin (1927) have confirmed the findings of Collip, and also remarked that the first areas of bloody exudation seemed to be confined to the fundic region of the stomach. Hueper (1927) has made microscopic studies of the gastric mucosa and of other tissues, and has substantiated and extended our observations.

In view of the above described findings, and because of the fact that the fundic region is the source of the hydrochloric acid of gastric juice, we were interested to determine the effects of parathyroid hormone upon gastric secretion. We reasoned that, following elevation of the blood calcium by the action of the hormone, there might arise a definite change in the gastric secretory mechanism before the fatal onset of the vomiting and hemorrhage just described. We thought that this functional change might be evidenced by changes in the responses to stimuli. If such a functional change could be measured one would have a second method of avoiding serious complications of overdosage added to the method of careful control of the blood calcium level. The present communication deals with some attempts to determine the influence of the parathyroid hormone upon gastric secretion. At the same time we wish to offer some data on the calcium concentration of the gastric juice, obtained from normal and hypercalcemic animals.

Experimental procedure. Female Pavlow pouch dogs were chosen for

experimental work. Dr. T. E. Boyd, of the Department of Physiology of this school, kindly placed four such animals at our disposal. For several days before the injection of the hormone the gastric secretion in response to a subcutaneous injection of 0.5 or 1.0 mgm. of histamine dihydrochloride dissolved in 1.0 cc. of physiological saline, was determined. We measured volume and acidities of the juice obtained prior to the injection for one hour to determine that the pouch was not under the influence of active stimuli. After the injection of the histamine we collected the juice for 45 minutes or one hour, depending on the size of the injected amine dose. Upon these samples we determined volume and acidities, the latter being calculated to clinical units.

In some cases the calcium content of the clear juice was determined in the following way. Two cubic centimeter portions of the juice were made slightly alkaline to phenolphthalein with calcium-free N/40 sodium hydroxide, and treated with 1 cc. of saturated ammonium oxalate, without any dilution other than that of neutralization. The completion of the analysis was made in the same manner as for blood plasma from this stage. Before injecting the hormone the calcium content of the blood of the normal animal was determined by the Tisdall (1923) method, using 2 cc. of heparinized plasma. During these control days, when the animals were normal, it was not thought necessary to determine the blood calcium level more than once, for it is known that the fluctuations are within narrow limits for the normal dog. Accordingly, values for blood calcium concentrations are not recorded in some of the control experiments.

The purified histamine dihydrochloride was given to us by Dr. Milton T. Hanke, of the Sprague Memorial Institute. Keeton, Koch, Luckhardt (1920) and Popielski (1920) have shown independently that the subcutaneous injection of a solution of histamine dihydrochloride stimulates the production of gastric juice, which is uniform with respect to volume and acidities, from the Pavlow pouch animal. Since 1920 other workers have found that histamine and its salts are valuable for testing the gastric function (Lim, 1924) (Gompertz and Vorhaus, 1925).

The following description of one of our control experiments will serve to illustrate in a general way the routine involved.

On 6–28–26 the secretion from normal animal 2 was collected from 9:45 to 10:45 a.m. The sample measured 2.0 cc. and had 17.0 free and 47.5 total acidities. At 10:45 a.m. injected 0.5 mgm. of histamine salt in a volume of 1.0 cc. of normal saline. The secretion from 10:45 to 11:30 a.m. was 18.7 cc. with 111 free and 124 total acidity. This juice contained 5.72 mgm. of calcium per 100 cc. The next volume secreted between 11:30 and 12:45 was 4.5 cc. with 81 free and 101 total acidity. Sixty units of hormone were injected at 1:45 p.m. and 75 units at 4:25 p.m.

The injection of the hormone, described above, marked the conclusion of the control days of study. The routine was little changed in the ex-

perimental days following the beginning of the blood calcium elevations, as the following record, extracted from the protocol of dog 2 on the day after the one above described, shows.

At 9:50 a. m. the plasma calcium of dog 2 was 17.67. The secretion from 10:43 to 11:43 was 2.0 cc. with no free and 20 total acidity. The heart rate had dropped from the normal of 120 to 84. The animal appeared less lively. At 11:45 injected 1 cc. of histamine solution containing 0.5 mgm. of the salt. In the next 45 minutes collected 11.0 cc. of juice, with 110 free and 120 total acid. The next 1¼ hours' volume was 3.2 cc. with 110 free and 113 total acid. The 11.0 cc. of juice had 5.92 mgm. of calcium per 100 cc. The injection of the histamine here caused the heart to accelerate from 84 to 120 within 15 minutes, but it slowed to 90 within the next 30 minutes.

For dogs 1 and 2 the amount of the histamine salt used to provoke secretion was 0.5 mgm., while 1.0 mgm. was used with each injection of animals 3 and 4. No food was given during the three-hour periods of observation. Dogs 1 and 2 received no water during the three-hour periods of study, while animals 3 and 4 received at will not over 125 cc. during each of the periods used.

The hormone preparation used was parathormone, Collip, (Eli Lilly & Co.) and was injected subcutaneously. The large amounts of this preparation used caused the deaths of animals 1 and 2, while animals 3 and 4 survived and have shown no ill-effects after the return to the normal blood calcium level.

Results obtained. Using dog 1 we made two preliminary control studies on the response of the normal animal to histamine injection. We then injected the hormone and caused elevation of the blood calcium and then measured the response of this animal to the same amount of histamine in 11 periods of testing. These results are summarized in table 1. With animal 2 we recorded three control periods and three responses of the same animal made hypercalcemic by hormone injections. The results are shown in table 2. With dog 3 we made three control tests of response to histamine and two tests on the hypercalcemic animal, followed by a single control test made after the blood calcium had decreased to normal. These results are summarized in table 3.

With normal animal 4 we have tested the response for four periods, then injected the hormone, and followed with four experiments on the same animal made hypercalcemic. These were followed by one control test after the effects of the hormone had disappeared in so far as the blood calcium level would show. These results are presented in table 4.

The histamine injection apparently did not influence the level of the blood calcium. This was seen in a record of dog 1, which was injected with histamine (0.5 mgm.), when the blood calcium was 17.48 mgm. in 100 cc. Fifteen minutes later the blood calcium value was 16.98 mgm. in 100 cc. The change noted is within the limits of error of the method

of experimentation, since the slight deviation may have been due to error of estimation or to the slight change of the calcium level with disappearance of hormone influence.

Each one of the animals used showed a slowing and irregularity of heart action, after hormone treatment, as described in our previous paper. The histamine injections caused accelerations in every instance noted. In one instance the histamine injection caused an acceleration of the heart of hypercalcemic dog 2 from 84 to the normal of 120, and in another obser-

TABLE 1

Influence of parathyroid hormone on gastric secretion

Studies on dog 1, weighing 12 kilos

DATE	VOLUME OF JUICE	ACIDITY		CALCIUM IN 100 CC. OF	
		Free	Total	Juice	Blood plasma
	cc.			*mgm.*	*mgm.*
6–12–26	19.0	110	129		
6–14–26	10.5	100	119		
6–15–26					11.26
6–16–26	5.5	63	90		17.58
6–17–26	7.0	56	95	8.59	17.96
6–18–26	8.5	110	133		18.55
6–23–26	3.3	25	50	5.63	13.03
6–24–26	5.4	100	123	5.23	
6–25–26	3.3	70	97		12.64
6–26–26	1.2	23	69		18.66
6–27–26	8.2	114	128	5.23	21.72
6–28–26	4.8	113	129	6.02	18.56
6–29–26	1.5	23	43		17.48
6–30–26	1.5	4	73		13.33
7– 1–26	0.0				11.55

This animal received injection of 50 units of parathormone at 9:20 a. m. and 50 more at 3 p. m. on 6–15–26. No more was given until 6–25–26, when 100 units were injected. The animal showed no signs of vomiting or hemorrhage until the morning of 7–1–26, and died during that day. On autopsy the pouch was found to have a bloody area of the entire mucosa, with the exception of the portion close to the scar of operation, which was remarkably normal in appearance.

vation from 78 to 120. The injection of animal 3 in one instance caused the acceleration from 90 to 162, while the heart rate of animal 4 was increased from 90 to 120. The heart rate usually increased within 15 minutes after the histamine injection, but usually slowed again within one hour after the injection.

Discussion. On first examination of the results of experiments with animal 1 it would appear that the secretion was decreased in volume and acidity after the injection of the hormone had elevated the blood calcium.

We are inclined to attribute this decreased series to the fact that the hormone causes some dehydration and to the fact that this animal received no water during any of the three hour periods of observation. Collip (1926) has shown that the injection of the hormone in large amounts causes a reduction in blood volume and a pronounced diarrhea, while Ivy (1918) and Sutherland (1921) have shown that water drinking with meals causes increases in volume and acidity of the gastric juice. On one day (6–23–26) this animal, 1, had a blood calcium nearly normal but had probably ex-

TABLE 2

Influence of parathyroid hormone on gastric secretion

Studies on dog 2, weighing 12.2 kilos.

DATE	VOLUME OF JUICE	ACIDITY		CALCIUM IN 100 CC. OF	
		Free	Total	Juice	Blood plasma
	cc.			*mgm.*	*mgm.*
6–26–26	12.0	96	115		11.06
6–27–26	10.0	95	111	6.02	
6–28–26	18.7	111	124	5.72	
6–29–26	11.0	110	120	5.92	17.67
6–30–26	12.5	118	131	5.53	18.85
7– 1–26	2.0				15.20

This animal received 60 units of parathormone at 1:45 p. m. and 75 units at 4:25 p. m. on 6–28–26 and no more until 6–30–26, when 100 units were given at 4:00 p. m. Bleeding from the pouch began the morning of 7–1–26 and the animal died at 11:55 a. m. The autopsy findings were the same as those upon dog 1.

TABLE 3

Influence of parathyroid hormone upon gastric secretion

Studies on dog 3, weighing 9.5 kilos.

DATE	VOLUME OF JUICE	ACIDITY		CALCIUM IN 100 CC. OF	
		Free	Total	Juice	Blood plasma
	cc.			*mgm.*	*mgm.*
9–16–26	22.0	120	133	6.84	
9–18–26	30.5	105	128	6.95	
9–20–26	14.0	103	115	6.65	
9–22–26					9.92
9–23–26	27.5	93	108	8.44	14.39
9–25–26	25.5	98	110	8.04	11.61
9–29–26	19.0	73	103	6.35	9.72

This animal received an injection of 100 units of parathormone at 4:45 p. m. on 9–20–26. No more was given and the animal made an uneventful recovery to the normal blood calcium level, and has since been used and shown a normally active pouch in other work.

perienced a large measure of dehydration due to the more active hormone influence on previous days. On this particular day the sample of juice measured only 3.3 cc. and was found to have free and total acidities of only 25 and 50 respectively. On the following day (6–24–26) when the blood calcium was probably between 13.03 and 12.64 mgm. per 100 cc., the animal showed a greater response of secretion of more juice with greater acidity. This animal showed the largest response in volume and acidity when the blood calcium was at its highest level in the times of observation. If the water intake is limited and the water loss is increased the stomach appears to form less juice and less acid for secretion.

Animal 2 received no water during the three hour periods of observation but the duration of the hypercalcemia and hormone influence, and pre-

TABLE 4

Influence of parathyroid hormone on gastric secretion

Studies on dog 4, weighing 11 kilos.

DATE	VOLUME OF JUICE	ACIDITY		CALCIUM IN 100 CC. OF	
		Free	Total	Juice	Blood plasma
	cc.			*mgm.*	*mgm.*
9– 9–26	37.0	115	135	4.81	
9– 9–26	35.0	115	128	4.86	
9–11–26	35.0	112	131	5.36	
9–14–26	34.0	115	130	4.96	12.80
9–15–26	30.0	115	132	6.35	19.85
9–16–26	39.0	115	128	6.35	18.06
9–18–26	20.0	110	123	5.25	13.50
9–20–26	25.0	108	123	4.86	12.31
9–23–26	22.5	100	112		10.32

This animal received 50 units of the hormone at 2:00 p. m. and 50 units at 4:30 p. m. on 9–14–26. No more was given and the animal made an uneventful recovery to the normal blood calcium level, and has since been used and shown a normally active pouch in other work.

sumably the measure of dehydration, was much less. The results with this animal indicate a normal response to histamine when the blood calcium was elevated by hormone influence. In view of the measurements with animals 1 and 2, and subsequent use of animals 3 and 4, we believe that the hormone influences the gastric secretion only indirectly by influencing the water balance of the body.

Animals 3 and 4 received 100 to 125 cc. of water during the three-hour period of observation. The tabulated results show that the volumes and acidities of the juice samples collected from these two animals after they had been made hypercalcemic were, within experimental error and physiological variation, as great as the corresponding measurements obtained from the same animals before hormone treatment.

The action of injected histamine in accelerating the slow hypercalcemic heart may be due in part to an alteration of the viscosity of the blood followed by an adjustment of the heart rate. Or the acceleration may be due to an adjustment of the heart rate to the fall in blood pressure, after dilatation of the capillaries by the injected amine.

We are inclined to believe that these animals which were receiving almost daily injections of histamine were not as depressed as animals previously under hormone influence and not treated with histamine. Animal 4 at one time showed a blood calcium of 19.55 mgm. per 100 cc., and recovered uneventfully, while animals used in previous work usually died after the blood calcium had been elevated to 15 or 18 mgm., without histamine injections during hypercalcemia. In the light of these observations histamine commends itself as a drug which may, to some extent, be found to offset the poisonous effects of overdosage with the hormone.

II. The calcium content of gastric juice. No recent values are available on the composition of the pure gastric juice in amount of calcium. This is partly explainable by the fact that methods of measuring small amounts of calcium are very recent, and by the fact that animals of this type are desired to supply juice without admixed food. Our determinations were made upon the alkalinized samples of pure juice, without any ashing process. It is possible that the values upon ashed samples would be slightly different, and we present the values in tables 1 to 4 with this in mind. The average of 11 samples of gastric juice of the dog with normal blood calcium was 6.08 mgm. of calcium per 100 cc. of juice. We have recorded the calcium concentrations of 12 samples of juice obtained from hypercalcemic animals. These values average 6.65 mgm. per 100 cc. of juice, although it appeared in some of the individual series of experiments that the calcium concentration of juice samples may have been elevated with increases of blood calcium. The range of the calcium concentrations observed was 4.81 to 8.59, but the majority of analyses showed values of 5 to 6.5.

In this connection it is of interest to note that Cameron and Moorhouse (1925) have compared the calcium concentrations of the blood and spinal fluid. They reasoned that the spinal fluid calcium was derived from that of the blood by dialysis. The spinal fluid calcium was about 53 per cent of the concentration which existed in the blood. From these observations they thought that about 53 per cent of the total blood calcium was normally present in the diffusible ionic form. Using animals with normal blood calcium concentration we have calculated that the calcium content of gastric juice was 55 per cent of that of the blood. While our determinations on gastric juice correspond closely with those of Cameron and Moorhouse on spinal fluid calcium, we do not know that the calcium of gastric juice is derived from that of the blood by a process of dialysis. On the other hand, we have noted that the calcium concentration of gastric

juice is not elevated when the blood calcium level is increased, although the blood calcium increase is, in part, either ionic or readily available. This last statement is supported by the observations in our previous paper (1927) where it was noted that magnesium sulphate injections were less toxic to the hypercalcemic than to the normal animal. We believe that the data given by us would not support any theory that the gastric juice calcium is a measure of the dialyzable or ionic calcium of the blood.

It would seem desirable to determine the calcium content of gastric juice obtained from animals after the fall in blood calcium produced by removal of the parathyroids. It is possible that the appearance and concentration of calcium in gastric juice may be dependent on a threshold value which may vary within a comparatively small range.

CONCLUSIONS

1. If the parathyroid hormone influence is severe and prolonged, and the water balance of the body is unfavorable because of restricted intake and increased elimination of water, the gastric response of the dog to histamine may be decreased.

2. If the parathyroid hormone is administered in large amounts, and the water balance of the body more favorably maintained, the gastric response of the dog to histamine is normal.

3. After overdosage of the dog with parathyroid hormone the Pavlow pouch continues to secrete until the beginning of hemorrhage, when it stops completely and suddenly.

4. The calcium content of pure gastric juice of the normal dog is 5 to 6.5 mgm. in 100 cc.

5. The calcium content of gastric juice of the dog is not greatly influenced by administration of the parathyroid hormone in amounts sufficient to elevate the blood calcium to the level of 18 or 19 mgm. in 100 cc.

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CONCERNING THE EFFECT OF COBALT ON INSULIN HYPOGLYCEMIA IN RABBITS[1]

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Bertrand and Mâcheboeuf found that the pancreas is one of the richest organs of the body in cobalt and nickel and that these metals passed into preparations of insulin (1). This observation encouraged them to determine whether these metals had any effect upon insulin hypoglycemia in rabbits (2). Their experiments indicated that injection of the metals intensified the hypoglycemia produced by insulin. This was usually shown not so much by an increased hypoglycemia as by a prolongation of it. Cobalt appeared to be somewhat more effective than nickel. The metals when injected in the same doses given with the insulin had no effect upon the blood sugar. These conclusions were based upon the results obtained from different animals. Our experience indicates that the insulin hypoglycemia resulting from a certain dose per kilo of body weight varies greatly from rabbit to rabbit. It seemed that the results obtained by the French investigators might have been due to individual variations and not to the metals in question. We have repeated these experiments using our standardized rabbits maintained under the strict regimen already described (3). The cobalt and insulin injections were given both intravenously and subcutaneously. The same preparation of insulin was used in all the experiments reported in this paper. The cobaltous nitrate administered was contaminated with 0.26 per cent nickel.

Intravenous experiments. The insulin was diluted 1 to 100 for injection; 0.7 cc. per kilo was the minimal convulsion dose when given intravenously. One cubic centimeter of insulin plus 1 cc. of a cobaltous nitrate solution were made to 100 cc. Injection of this solution containing 0.02 mgm. cobalt in 1 cc. resulted in the same value for the convulsion dose. A straight metallic solution containing 0.041 mgm. cobalt per cubic centimeter had no effect on the blood sugar when given in doses of 1 cc. per kilo body weight.

Five rabbits were used in the remaining intravenous experiments. In

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TABLE 1

Influence of cobalt and insulin injected intravenously

DATE	RABBIT NUMBER	WEIGHT	INTRAVENOUS FACTOR	INSULIN PER KILOGRAM	COBALT PER KILOGRAM	BLOOD SUGAR			REMARKS
						Before	1.5 hours after	3 hours after	
1926		*kgm.*		*cc.*	*mgm.*	*mgm. per 100 cc.*			
September 30	97	3.15	1.66	0.9	0.009	93	28	71	Insulin diluted 1:200
October 7	97	3.00	1.66	0.9		87	40	66	
September 30	98	2.83	1.66	0.9		100	33	57	
October 7	98	3.00	1.66	0.9	0.009	85	40	66	
September 30	64	3.30	1.16	0.9		100	80	114	
October 7	64	3.30	1.16	0.9	0.009	80	100	105	
October 1	19	3.35	1.16	0.9	0.009	105	65	80	Co given 1 hour after insulin
October 8	19	3.50	1.16	0.9		125	85	111	NaCl given in place of Co
October 1	37	3.50	1.16	0.9	0.009	118	62	93	Co given 1 hour after insulin
October 8	37	3.50	1.16	0.9		138	85	121	NaCl given in place of Co

TABLE 2

Influence of cobalt and insulin injected subcutaneously

DATE	RABBIT NUMBER	WEIGHT	INTRAVENOUS FACTOR	INSULIN PER KILOGRAM	COBALT PER KILOGRAM	BLOOD SUGAR			
						Before	1.5 hours after	3 hours after	5 hours after
1926		*kgm.*		*cc.*	*mgm.*	*mgm. per 100 cc.*			
October 14	98	3.00	1.66	0.6	0.06	108	50	40	
October 21	98	2.80	1.66	0.6		111	55	77	74
October 14	64	3.40	1.16	0.6	0.06	87	43	48	
October 21	64	3.40	1.16	0.6		105	62	43	91
October 28	64	3.40	1.16	0.6		95	55	67	95
November 4	64	3.50	1.16	0.6	0.06	121	91	100	85
October 15	19	3.60	1.16	0.4	0.04	125	65	87	118
October 22	19	3.50	1.16	0.4		111	52	87	100
October 29	19	3.50	1.16	0.4	0.04	108	83	87	103
November 5	19	3.50	1.16	0.4		118	63	80	95
October 15	37	3.50	1.16	0.4	0.04	125	61	100	118
October 22	37	3.50	1.16	0.4		118	57	74	105
October 29	37	3.50	1.16	0.4	0.04	138	80	91	121
November 5	37	3.60	1.16	0.4		118	77	77	111

Insulin diluted 1 to 200.

these the blood sugar values were estimated before the injection and 1.5 and 3 hours afterward. The blood sugar for each animal was determined for insulin with and without the addition of cobalt. The results are given in table 1. The actual amounts of insulin and cobalt given may be calculated by multiplying the weight × the dose × the intravenous factor. The findings are summarized by saying that rabbits 97, 19 and 37 showed a somewhat greater hypoglycemia when cobalt was administered. Rabbit 98 had a more profound hypoglycemia without the cobalt. The results for rabbit 64 were inconclusive because the dose of insulin was too small to produce a large enough fall in the blood sugar.

Subcutaneous experiments. The results of the subcutaneous experiments are given in table 2. The added cobalt appeared to enhance the hypoglycemic action of the insulin in but one (no. 98) of the four rabbits.

The experimental findings indicate that cobalt is without appreciable influence on the insulin hypoglycemia in rabbits.

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THE REGULATION OF RESPIRATION

IX. The Relation of Tissue-Acidity and Blood-Acidity to Volume-Flow of Blood as Illustrated by Hemorrhage and Reinjection

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It is not an uncommon occurrence for pulmonary ventilation to increase with hemorrhage. As a rule the greater the hemorrhage the greater is the ventilation. But such results are by no means constant, for the conditions under which hemorrhage occurs exercise modifying influences. For example, hemorrhage in the normal, intact, unanesthetized dog, perhaps by virtue of its quieting effects, may for a time decrease the rate of breathing and the total amount of air moved (Gesell, Blair and Trotter, 1922). On the other hand—in an acute experiment with relatively light anesthesia increased pulmonary ventilation commonly results (Gesell, Capp and Foote, 1922). Again—with heavier narcosis the incidence and degree of increased ventilation decrease with bleeding, and depression becomes more frequent (Bald, 1927).

It is not the purpose of this paper to attempt a detailed explanation of the variations in response to hemorrhage under varying conditions, but rather to elucidate the relation of the behavior of the respiratory center to volume-flow of blood under the particular conditions of these experiments.

Granting the rôle of the metabolism of the respiratory center in the control of pulmonary ventilation (Gesell, 1923, 1925; McGinty and Gesell, 1925) and granting a high metabolic rate of the center (McGinty and Gesell, 1925), two disturbances resulting from change of blood-volume call for attention. For a reduction of volume-flow of blood not only impairs oxidations (Gesell, Foote and Capp, 1923) but alters the acid production as well. The normal formation of carbonic acid theoretically gives way (Hill, 1922; Meyerhof, 1921, 1922) to a greater formation and accumulation of lactic acid. Add to this a hampered removal of acid resulting from a broken coördination of the dual function of hemoglobin[1] and conditions

[1] To be sure there is no broken coördination of the dual function of hemoglobin in the passage of blood through the lungs during hemorrhage for the oxidative change is considerably greater than normal. Not only does the blood arrive at the lungs more

favorable for increased acidity of the tissues obtain. Agreeing with this conception, hemorrhage was shown to increase the response of the dog to the administration of carbon dioxide (Gesell, Capp and Foote, 1922). Increased pulmonary ventilation during hemorrhage is, therefore, conceivably due to an increased acidity of the respiratory center. For the elaboration of this problem we have followed changes in acidity in the arterial and venous blood during hemorrhage and reinjection.

METHOD. Continuous records of changes in acidity of the circulating blood were made with the manganese dioxide electrode (Gesell and Hertzman, 1926) and checked with the hydrogen electrode. The arterial electrode was placed in the carotid artery, and the venous electrode in the external jugular vein of the opposite side. Room air was administered and ventilation and oxygen consumption were recorded with a rebreathing tank. In some experiments artificial ventilation was administered by placing the pump in closed circuit with the rebreathing tank. Blood pressure was recorded with the mercury manometer and time was registered in one- and six-second intervals. The horizontal bar is equivalent to one minute.

RESULTS. About thirty experiments were done. A few observations from eight of these appear in figures 1 to 6. In figures 1, 2A, B and C, 3A, 5A, B, C and E the chest was closed and pulmonary ventilation was under the control of the animal. In the remaining experiments pneumothorax was established and uniform ventilation supplied by artificial means. Hemorrhage was varied in amount and the period of depleted blood-volume was varied in duration. These constituted the main variables.

Before analyzing the results obtained with the manganese dioxide electrode it should be recalled that the method used is not a quantitative method—that its value lies in continuity of record, gross information on directional changes of acidity, accuracy of time relations and abundance of data. The electrode, like other electrodes, or other methods for determining hydrogen ion concentration, is sensitive to more than one chemical influence. A highly complex variable system such as the circulating blood, which may be modified at any moment by the metabolism of the tissues, demands a scrutiny of the behavior of the electrode under the particular condition of its use. The "alkaline" effect of intravenous injection of dextrose was previously explained by a direct reducing effect of sugar on the electrode (Hertzman and Gesell, in press). The disagreement with the quinhydrone and hydrogen electrodes following massive injection of sodium

reduced than normal, but oxidation is more complete. And in the tissues the blood which arrives more oxidized than normal is reduced to a lower level than normal. Yet it would appear that the coördination of the dual function of hemoglobin is broken, for the total oxygen consumption is reduced by hemorrhage. Presumably the total amount of acid formed is in excess of the total amount of alkali liberated in the reduction of oxyhemoglobin.

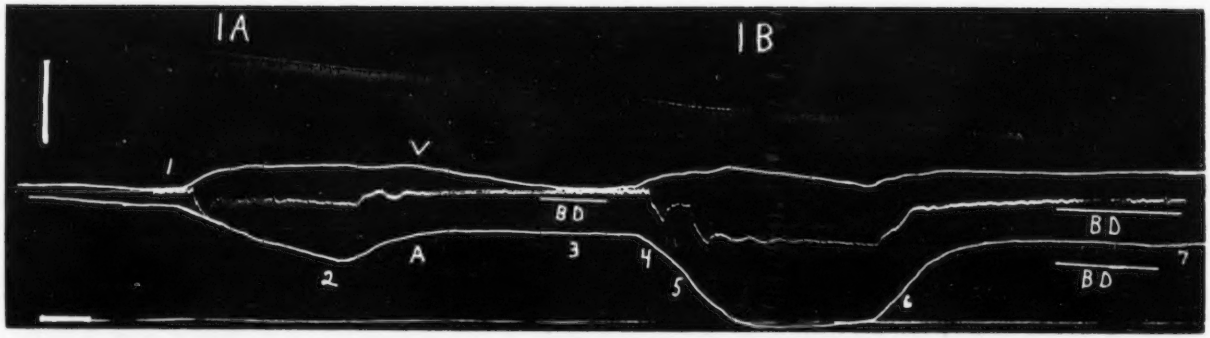

Fig. 1, A and B. Hemorrhage and reinjection during normally controlled pulmonary ventilation. Dog 12 kgm. body weight. Upper record—pulmonary ventilation and oxygen consumption. *V*—venous acidity curve (external jugular vein). *A*—Arterial acidity curve (carotid artery). Upstroke indicates increasing acidity. The vertical bar is equivalent to approximately 0.1 pH change. Mean blood pressure is recorded with the mercury manometer. Time is registered in seconds. One minute is indicated by the horizontal bar. *B. D.*—extension of the basal or alkaline drift. *1*—97 cc. hemorrhage (0.88 per cent of body weight or 9 per cent of total blood-volume). *2*—97 cc. reinjection. *3*—see text. *4*—100 cc. hemorrhage. *5*—100 cc. hemorrhage. *6*—200 cc. reinjection.

cyanide was explained by the liberation of excessive amounts of reducing substances by the tissues into the blood (Gesell and Hertzman, 1926). (Further work on this point will be published later.) The occasional alkaline swing of the record at the end of a long period of mechanical asphyxia (not reported) is probably a similar phenomenon. Excessive production of reducing metabolites during hemorrhage must then be admitted as a probability.

Directional agreement with the quinhydrone electrode during hemorrhage has been reported (Gesell and Hertzman, 1926), and the recent results of Bald (1927) with the colorimetric method agree with the findings with the manganese dioxide electrode. But until the validity of the use of the quinhydrone electrode (which is also an oxidation-reduction system) is established for severe conditions of anoxemia in the acute experiment, further checks with the hydrogen electrode seemed a good precaution. Examples of comparison of acidity changes as recorded by the manganese dioxide and hydrogen electrodes appear in figures 5A, B, C and D.

In analyzing these records it is recalled that the manganese dioxide electrode never comes into complete equilibrium with the surrounding blood though the reaction of the blood remains the same. This lack of equilibrium is shown in the "alkaline" drift which varies with the animal and with the duration of the equilibration of the electrode with the blood. The longer the equilibration the less steep is the drift gradient. In comparatively long observations (fifteen minutes or more) the change in drift becomes an appreciable factor—particularly if the electrodes are poorly equilibrated.

Compare the dotted curve established with the hydrogen electrode and the manganese dioxide record. In figures 5A, B and D arterial records are checked, and in figure 5C a venous record is checked. The agreement in these and other records not published is on the whole very good. Highly quantitative agreement is not to be anticipated. The results may always be colored by reduction, for they unmistakably appear when hemorrhage is severe. See, for example, figure 5E. Here conditions for serious impairment of oxidation obtained. Depleted blood-volume was maintained dangerously near to death. Respiration had already stopped and improvement from reinjection was slow. Towards the close of recovery the arterial blood is abnormally "alkaline" and the venous blood only slightly more acid than normal. This distortion of the record may be due to a liberation of reducing metabolites into the circulating blood.

It will then be assumed that the effects of reducing substances are relatively great only under severe conditions,—that under the conditions of hemorrhage as here presented the manganese dioxide electrode is sufficiently specific to establish the principles involved in this research. The comparison of the manganese dioxide and hydrogen electrode curves

indicates that this assumption is valid. For purposes of simplicity and clearness of description changes in acidity will be referred to in terms of pH.

In figure 1 a dog weighing 12 kgm. was bled 97 cc. (0.88 per cent of the body weight or 9 per cent of the total blood-volume), and three minutes later the blood was reinjected. The venous acidity curve is above and the arterial acidity curve below. On hemorrhage the mean blood pressure dropped only 6 mm. Hg (from 144 to 138 mm.). Pulmonary ventilation increased. The period of depleted blood-volume was too short for accurate determination of changes in oxygen consumption, but reduction is indicated. The arterial blood turned alkaline and the venous blood turned acid. On reinjection the arterial blood turned acid, the venous blood turned alkaline, and pulmonary ventilation diminished.

In extending the basal drift in figure 1[2] we find at *3* that the venous blood after five minutes of recovery was distinctly more acid than normal, *1*. The arterial blood was likewise more acid. Such results are confirmed by checks with the hydrogen electrode. They suggest an accumulation of acid in the blood. In figure 1B 100 cc. of blood were drawn at *4* and another 100 cc. at *5*. There was greater depression of oxidations, greater stimulation of ventilation, greater increased alkalinity of the blood, and greater initial increased acidity of the venous blood. The recovery acidity values for both the arterial and venous blood were considerably higher, agreeing with the magnitude of the circulatory and respiratory disturbance.

In figures 2A, B and C more striking effects of hemorrhage are shown. In record 2A 350 cc. of blood (2.54 per cent of the body weight or 29 per cent of the total blood) were taken in three samples. The large fall in blood pressure was undoubtedly accompanied by a large drop in the volume-flow of blood. The effects of this are seen in the enormous reduction of basal metabolism accompanied by a corresponding increase in pulmonary ventilation. Arterial blood increased in alkalinity approximately 0.2 pH (checked with the hydrogen electrode) and was still rapidly increasing at the end of the period of depleted blood-volume. Despite this large increased alkalinity of the arterial blood the venous blood returns from the tissues more acid than normal. Even more noteworthy—despite the progressively increasing alkalinity of the arterial blood the venous blood progressively turned more acid. Surely the tissues drained by the external jugular vein must have turned more acid as the arterial blood turned alkaline. In hemorrhage there can hardly be any doubt of this inverse relation. With reinjection the same striking changes occurred in the reverse direction. It seems equally reasonable to assume that the tissues turned alkaline as the arterial blood turned acid. Accordingly, in

[2] Correction for the progressively diminishing drift has not been made in any of the records. This should be taken account of in the interpretation of prolonged and shorter observations where the drift is large.

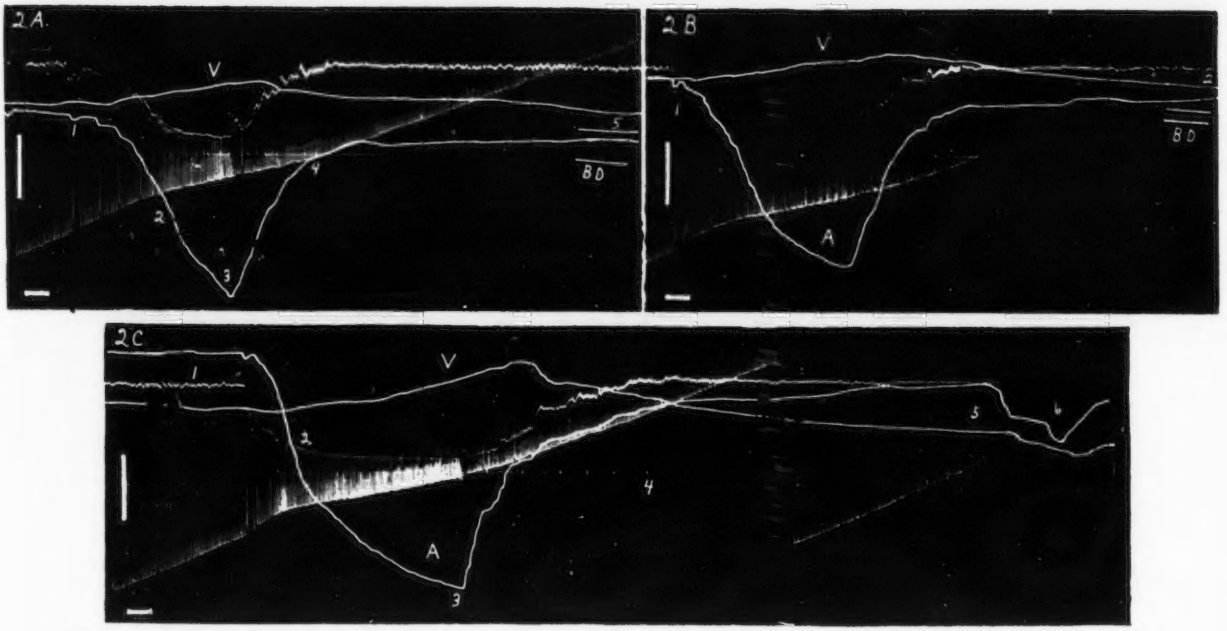

Fig. 2, A, B and C. Hemorrhage and reinjection during normally controlled pulmonary ventilation. Note that the record of pulmonary ventilation is reversed as compared with figure 1. A, *1* and *2*—350 cc. hemorrhage (2.54 per cent of body weight); *3* and *4*—350 cc. reinjection. B, *1*—400 cc. hemorrhage followed by reinjection. *C*, *2*—500 cc. hemorrhage; *3* and *4*—650 cc. reinjection; *5* and *6*—administration of liquid ether.

hemorrhage the acidity of the venous blood is a more reliable index to changes in acidity of the tissues than the acidity of the arterial blood. Extending the basal drift in figure 2A indicates again that both the venous and arterial blood at the end of recovery are more acid than before hemorrhage (approximately 0.05 pH).

In figure 2B hemorrhage was a little larger. In figure 2C it was 150 cc. greater. The period of depleted blood-volume was considerably prolonged. The venous blood at the end of recovery is not as acid as might be expected but this may be explained by the fact that the injection of blood at *3* was about 150 cc. in excess of the preceding hemorrhage. Presumably the volume-flow during recovery was then greater than before hemorrhage.

Of the host of factors influencing the hydrogen ion concentration of the circulating blood in hemorrhage two factors would seem to exert predominating effects—changes in pulmonary ventilation and changes in volume-flow of blood. Examples of these effects are separately shown in figures 3A and B.

Assuming that the blood is returning from the tissues at a constant rate of flow, the hydrogen ion concentration of the blood leaving the lungs will be determined by the degree of pulmonary ventilation. Since the hemoglobin is almost completely oxidized excess ventilation exerts primarily an alkaline effect. The effects of sudden change in ventilation are shown in figure 3A. Superimposed upon the usual increased alkalinity of the arterial blood from hemorrhage are several sudden increases in alkalinity followed by equally rapid increases in acidity—each caused by a deep respiration followed by a respiratory pause.[3] The efficiency of deep respiration is thus illustrated.

The other predominant factor is volume-flow of blood. With a constant pulmonary ventilation and uniform composition of the blood arriving at the lungs the degree of ventilation of this blood should be a function of the flow. The slower the flow the better its ventilation and the greater its alkalinity. Effects of changing flow of blood are shown in figure 3B, in which waves of blood pressure spontaneously developed after the administration of urethane. Artificial ventilation is constant. The changes in blood acidity are undoubtedly related to changes in volume-flow of blood accompanying the variations in mean blood pressure.

[3] It is not to be assumed, however, that the respiratory pause comes as an effect of decreased alveolar carbon dioxide tension. The chemical effects of this change occur only after the altered blood has reached the general circulation. Neither is the beginning of ventilation due to increased alveolar carbon dioxide tension, for that also appears too promptly. The administration of carbon dioxide increases ventilation only after the supersaturated blood reaches the general circulation. The respiratory pause is probably of reflex origin.

If this is correct the same directional changes in acidity should occur with hemorrhage and reinjection when pulmonary ventilation remains constant, as with hemorrhage and injection when pulmonary ventilation is normally controlled. Figures 4A, B and C and 5D show that they do. They stress the importance of volume-flow of blood in the determination of blood acidity, in the transport of acid and the control of the acid-base equilibrium of the body. Certainly if the blood coming from the tissues arrives at the lungs more acid than normal during hemorrhage and leaves the lungs more alkaline than normal even though the tidal air remains the

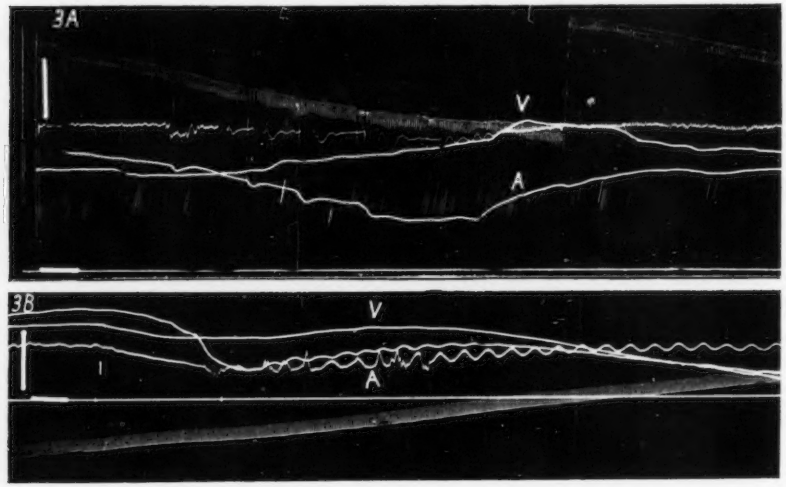

Fig. 3, A and B. A—effects of sudden changes of pulmonary ventilation resulting from deep breaths superimposed upon changes in acidity of the arterial blood produced by hemorrhage and reinjection. B—effects of variation of volume-flow of blood on the acidity of the arterial blood during administration of constant pulmonary ventilation.

same, the effect of retarded flow of blood is indicated. At least the conditions for a more extensive removal of carbon dioxide per unit flow of blood obtaining might well explain the findings. The same reasoning holds for the effect of flow of blood through the tissues. If the highly ventilated blood leaving the lungs during hemorrhage arrives at the tissues more alkaline than normal and leaves more acid than normal the effect of retarded flow in the tissues is demonstrated. Here, as in the lungs, the pH change occurring in the blood as it flows through the tissues is enormously greater than normal. This point is emphasized, for recent papers in the literature indicate that the effects of flow of blood are not fully appreciated.

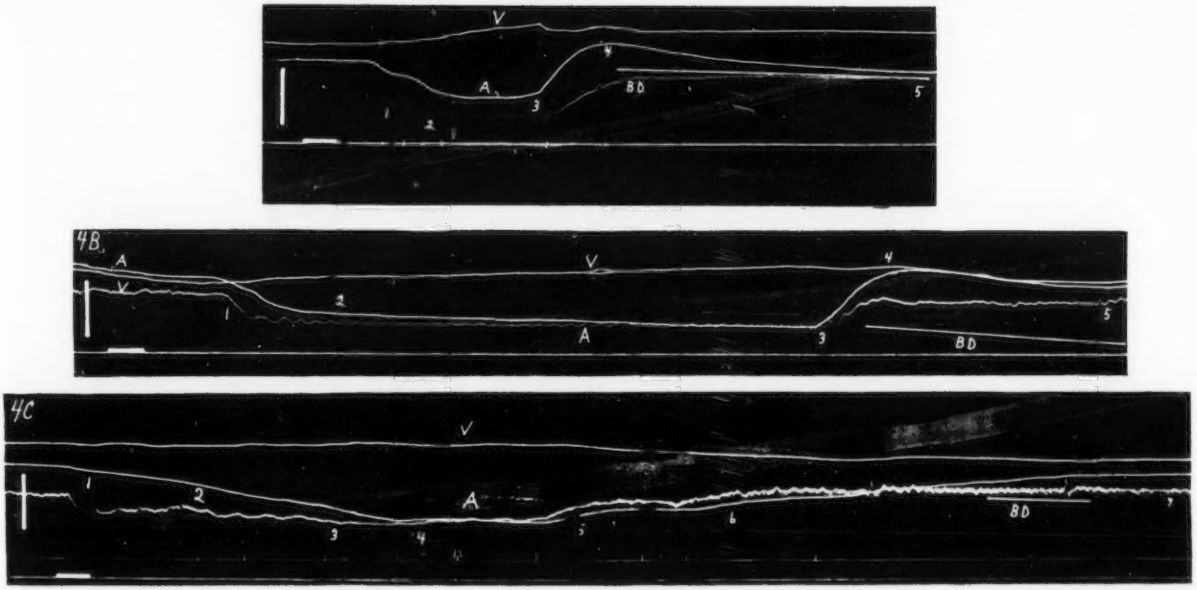

Fig. 4, A, B and C. Hemorrhage and reinjection during the administration of constant pulmonary ventilation with pneumothorax. A and B—dog 9 kgm. body weight. A, *1*—100 cc. hemorrhage; *2*—500 cc. hemorrhage; *3*—150 cc. reinjection. B, *1*—150 cc. hemorrhage; *3*—150 cc. reinjection. C—dog 11.5 kgm. body weight. C, *1*—100 cc. hemorrhage; *2*—70 cc. hemorrhage; *3*—70 cc. hemorrhage; *4*—70 cc. reinjection; *5*—100 cc. reinjection; *6*—70 cc. reinjection.

In figure 4A, illustrating the influence of volume-flow on blood acidity with artificial ventilation, the hemorrhage is of short duration, arterial overshooting is distinct and recovery is good. In figure 4B hemorrhage is long sustained. Correction for the basal drift indicates that the arterial blood has reached its maximum alkalinity approximately at *2*, and there began to swing acid. At *3* the normal acidity preceding hemorrhage has been reached. Reinjection at this point produces a marked increase in acidity of the arterial blood and a definite overshooting. Though the venous blood turns alkaline both recovery values are approximately 0.1 pH more acid than normal.

Figure 4C is taken from another dog weighing 11.5 kgm. Pulmonary ventilation is considerably in excess of normal. The animal was bled and injected at widely separated intervals; 100 cc. were bled at *1*, 100 cc at *2*, and 70 cc. at *3*. Seventy cubic centimeters were reinjected at *4*, 100 cc. at *5*, and the remaining 100 cc. at *6*.

Though the directional changes in blood acidity are similar with normal and artificially controlled ventilation, the overshooting of the arterial record in the latter represents a distinct difference. This is possibly explained by an accumulation of acid during hemorrhage which under more normal conditions would have been blown off. Increased volume-flow of blood from reinjection provides the usual increased acidity of the arterial blood; and the accumulated excess acid, until it is liberated, provides the overshooting. This is sustained by comparison of figures 4A and B and 6D with figures 1 and 2A and B. Though overshooting may occur in either type of experiment, its abruptness and extent bear a relation to previous ventilation.[4]

The question arises—how well do the changes in blood acidity here recorded indicate changes in acidity within the respiratory center? The arterial blood is a mixed sample, and the venous blood a local sample, affected only by the tissues through which it has flowed. In these experiments the external jugular vein was used on account of its size and position and free flow of blood, but unfortunately for our purposes it carries no

[4] The beneficial effects of ventilation will be emphasized in another paper comparing the effects of normal and constant artificial administration of air poor in oxygen. With constant ventilation the same overshooting occurs as is here found in hemorrhage.

Fig. 5, A, B, C and D. A comparison of the acidity curves of the manganese dioxide and hydrogen electrodes. The dotted curve is established with the hydrogen electrode. In figures 5, A, B and D, arterial acidity curves are compared and in figure 5C venous acidity curves. Constant ventilation is administered in figure 5D. Figure 5E shows the effects of extremely severe hemorrhage. The acidity records are colored by the action of reducing metabolites liberated by the tissues into the blood stream.

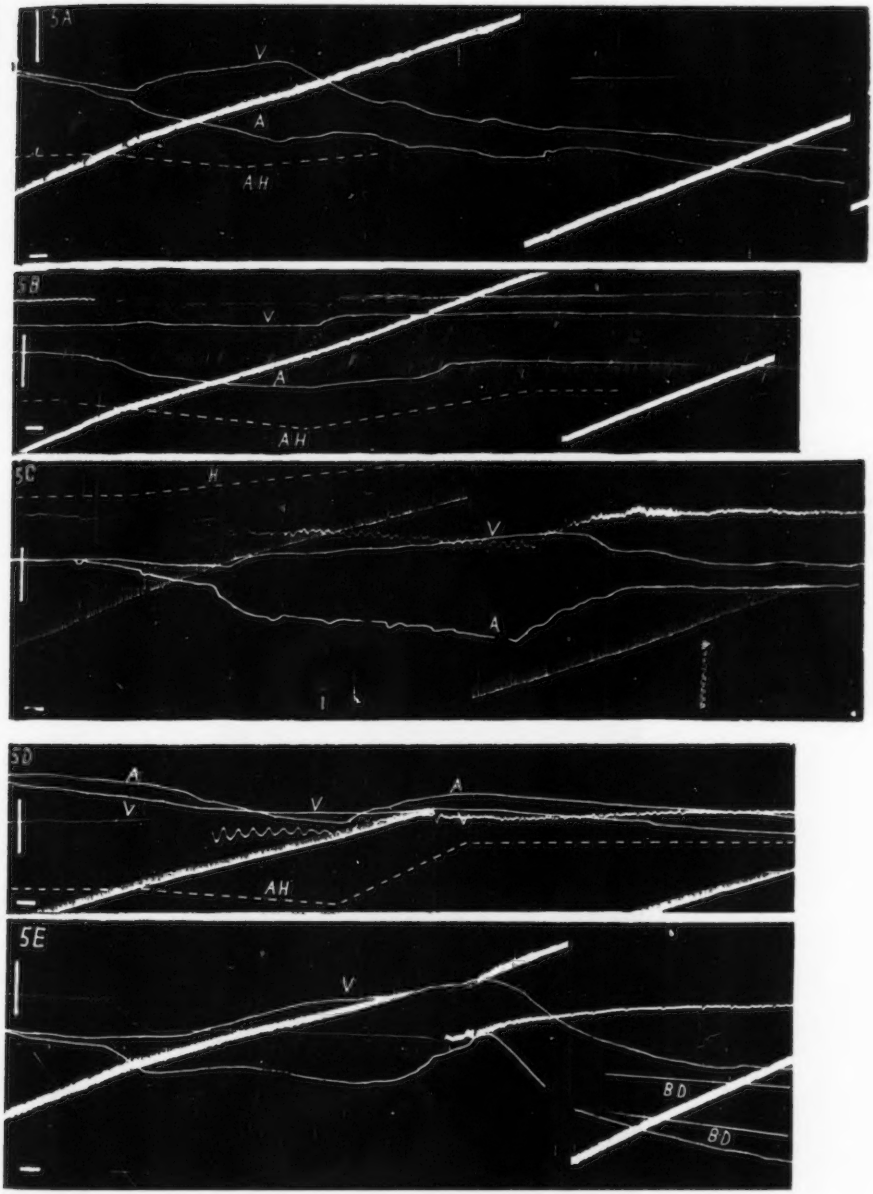

Fig. 5

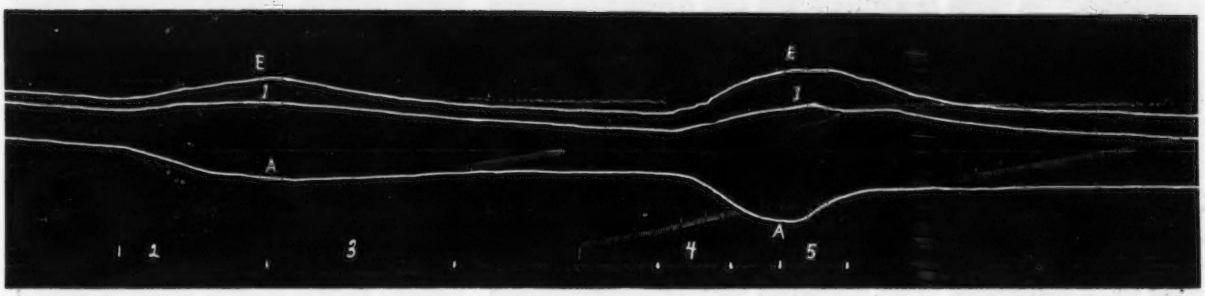

Fig. 6. Hemorrhage and reinjection during normally controlled ventilation. Comparison of internal and external jugular venous blood. Dog 10.2 kgm. body weight. E—external jugular acidity curve; *I*—internal jugular acidity curve; *A*—arterial acidity curve; *1*—75 cc. hemorrhage; *2*—75 cc. hemorrhage; *3*—150 cc. reinjection; *4*—220 cc. hemorrhage; *5*—reinjection.

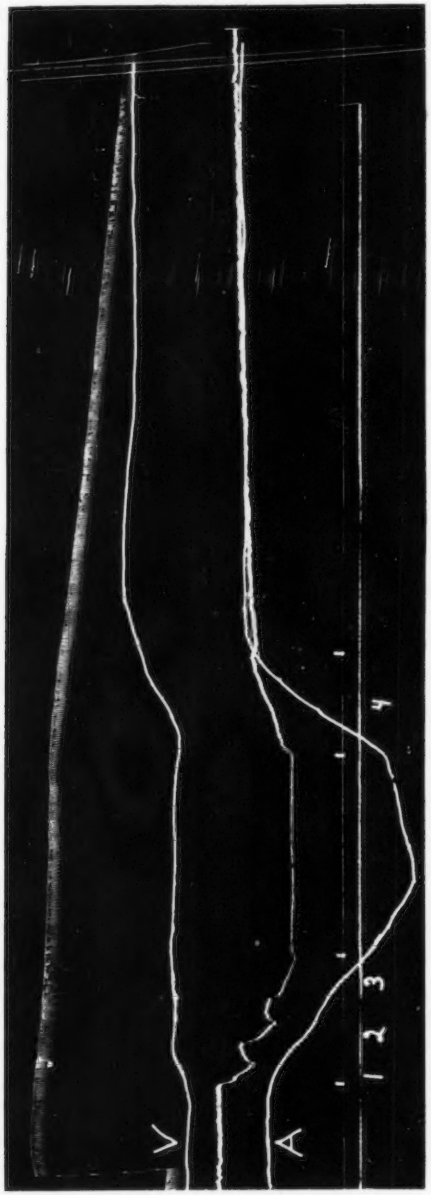

Fig. 7. Hemorrhage and reinjection with normally controlled ventilation. *1*—100 cc. hemorrhage; *2*—100 cc. hemorrhage; *3*—100 cc. hemorrhage; *4*—300 cc. reinjection.

blood from the brain. Obviously an electrode placed in one of the cerebral sinuses would provide more direct evidence. This, however, has not been done. Our nearest approach is a record of changes in acidity of the blood of the internal jugular (see fig. 6). The similarity of the external and internal jugular acidity curves support the use of the data obtained with the external jugular vein for the study of the acid-base equilibrium in the brain, though it must be remembered that the internal jugular vein carries blood from other tissues as well as from the brain.

In reviewing the figures of this paper it is worthy of note that on the whole the increase in alkalinity in the arterial blood is large and the increase in acidity in the venous blood relatively small. At times the venous blood changes very little in acidity, which is suggestive of the protective mechanism occurring on the pulmonary side to provide for the acid effects of slowed circulation on the tissue side. Exceptions to this more general finding occasionally occur. In figure 5A the acidity of the venous blood increased 0.15 pH, whereas the alkalinity of the arterial blood increased only 0.03 pH. Contrast this with figure 7, in which there is virtually no increase in acidity of the venous blood until reinjection. Then the increased acidity is well maintained.

Figure 7 illustrates other points of interest. It shows a stronger tendency than usual for the arterial blood to turn acid during the period of depleted blood-volume. This is undoubtedly due in part to the reduced pulmonary ventilation. On the venous side the initial decrease in acidity with reinjection is replaced by a rather large increase in acidity which is well maintained. To what extent the increased acidity is due to depressed ventilation during recovery and to a reduction of the alkaline reserve of the blood by fixed acids from the tissues was not determined. The record also shows the general tendency towards a greater increased acidity of the venous blood than of the arterial blood at the end of recovery. This tendency is seen in the other figures in the separation of the arterial and venous records. This is in line with the findings of these experiments—that decreased volume-flow increases the acidity of the venous blood and increases the alkalinity of the arterial blood, for the flow of blood at the end of an observation is presumably slower than at the beginning. An exception to this difference in flow is probably found in figure 3C, in which reinjection was 150 cc. in excess of hemorrhage. The figure fails to show the usual separation of the acidity records.

Discussion. It has not been our effort in these experiments to determine the minimum hemorrhage which would elicit increased ventilation, nor the minimum change in acidity of the blood associated with increased ventilation. Had that been our object, we should have used much lighter anesthesia. It is all the more remarkable that despite the absence of these precautions stimulation occurred as frequently as it did.

The primary object was to elaborate the chemical mechanism of control. By inference we are probably safe in concluding from the results of these experiments that hemorrhage increased the acidity of and reduced the oxidation in the respiratory center. The experiments, therefore, support the theory of acid control of respiration. On the other hand—the experiments give no evidence against the stimulating effect of lack of oxygen, unless we consider the depression of ventilation in figure 7 as such. The difficulty, as was suggested by one of us (Gesell, 1925,1926) of arriving at a clean-cut decision is the possibility of associated changes in acidity and oxidation. Reduced oxidations may lead to increased acidity in the tissues and increased acidity may lead to reduced oxidations. The experimental evidence, however, seems to give preponderating support to the acid mechanism of control, but granting the possibility of either factor controlling ventilation, the possibility of both factors working in the same direction must likewise be recognized. A critical test of this postulate is desirable. In the absence of such, a working hypothesis suggesting the possibility of a mechanism coördinating the effects of impaired oxidation and increased acidity has been proposed, (Gesell, 1926) merely as a guide to further experimental work. Though the hypothesis appears to be in agreement with many known facts, it is suggested that it be accepted at present only as a means of weighing evidence and arriving at a final solution.

SUMMARY

Effects of hemorrhage and reinjection on the acidity of the circulating arterial and venous blood were studied with the manganese dioxide electrode and checked with the hydrogen electrode.

Hemorrhage elicited increased alkalinity of the arterial blood and increased acidity of the venous blood. This was accompanied by decreased oxygen consumption and increased pulmonary ventilation. Reinjection increased the acidity of the arterial blood and decreased the acidity of the venous blood. This was accompanied by increased oxygen consumption and decreased pulmonary ventilation.

The same directional changes in blood acidity occurred with hemorrhage and reinjection during constant artificial ventilation.

The increased alkalinity of the arterial blood during hemorrhage under normal control of ventilation is, therefore, due not to increased tidal air alone, but to pulmonary flow of blood as well. The view of the importance of volume-flow of blood for the maintenance of normal acid-base equilibrium is thus supported.

The fact that venous blood more acid than normal leaves the lungs distinctly more alkaline than normal during constant pulmonary ventilation indicates the importance of flow in the pulmonary circuit.

The fact that highly alkalinized blood returns from the tissues more acid than normal indicates the importance of systemic flow of blood.

The results of these experiments agree with the common inverse relation between acidity of the arterial blood and respiratory movements. The relation between acidity of the venous blood and pulmonary ventilation was more direct.

The decreased oxygen consumption during hemorrhage and the excessive consumption following reinjection suggest increased production and accumulation of acid in the respiratory center during hemorrhage. The increased acidity of the venous blood suggests the same.

The findings here reported support the view of the importance of acidity in the control of pulmonary ventilation but they do not necessarily preclude other effects of altered oxidations.

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THE RESPONSE OF THE CIRCULAR AND LONGITUDINAL MUSCLE OF RABBITS' ILEUM TO PHYSICAL AND MECHANICAL CONDITIONS

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CIRCULAR AND LONGITUDINAL INTESTINAL MUSCLE. The presence in the intestines of two sheets of muscle fibers, arranged at right angles to each other, inevitably suggests the question of how these coöperate with each other in the complex movements of the intestines. The question can scarcely be answered until the contractile properties and responses of the two sets of muscles, separately and in correlation, have been studied and compared. This has been attempted by attaching levers in the direction respectively of the longitudinal and of the circular fibers. Bayliss and Starling (1899) for instance obtained simultaneous records from two enterographs placed at right angles on the intestine of the living animal. However, this arrangement shows only the *movements* in the longitudinal and in the transverse direction but, as will be explained, it does not differentiate the *share* that the two sets of muscles have in the movements. When Magnus in 1904 introduced his method of studying excised intestinal segments he apparently did not attempt simultaneous records, but suspended the segments either in the longitudinal or in the transverse direction. This also reflects only the direction of the movements, and does not differentiate between the two sets of muscles, unless very narrow strips of intestine are used.

In this earlier work, the comparison of the two muscular layers was merely incidental and rather subordinate to other problems. In 1920 and 1921 the question was attacked more systematically on excised intestine by Uhlmann and Abelin, and by Hideo Inoue. The latter split the segment so as to form a rectangle with the sides parallel respectively to the longitudinal and circular muscles. This rectangle was anchored at the center of two sides, with levers in opposition (the solid line of fig. 1, *I*). It was intended that each lever be moved by its parallel muscles. Uhlmann and Abelin cut an isosceles triangle, with its base parallel to the circular fibers of the split intestine, and arranged this as shown in the solid lines, figure 1, *II*.

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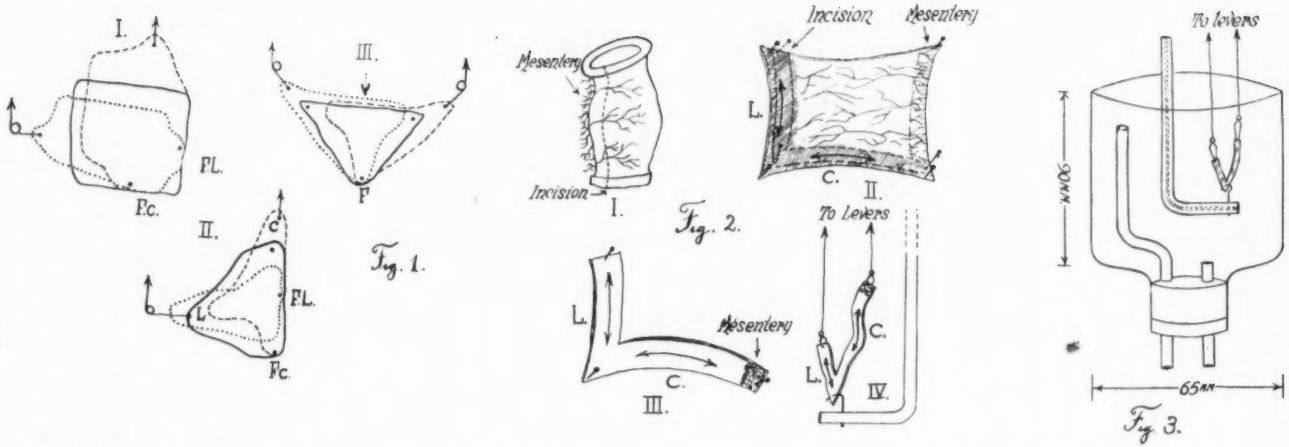


Fig. 1.

Fig. 2.

Fig. 3.

Fig. 1. Mutual distortion of the muscular coats of the intestine. The solid lines represent the original shape of the piece of open intestine, fixed by pins at the points marked *F*. The dotted lines show the shape after slight traction in the longitudinal, and the broken lines, in the circular direction. In *I* the piece is cut and arranged according to Inoue; in *II* according to Uhlmann and Abelin; *III* shows the triangle originally employed by the authors.

Fig. 2. Preparation of the L-shaped segments (see text).

Fig. 3. Arrangement of the saline bath.

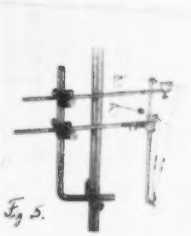
This has an advantage over the rectangle, in that it contains less superfluous muscle lying outside the lines of the pull; but it must hamper the circular muscle by fixing the center of the base and by unsymmetrical distortion through the longitudinal muscle. We thought to avoid these disadvantages by cutting a right-angled triangle from the intestine with its two sides parallel to the longitudinal and circular fibers. The right angle was used as fixed point, and the levers were attached to the two acute angles as shown by the solid lines of figure 1, *III*. This places the pull in the direction of the fibers and insures them symmetrical conditions; what is even more important, it eliminates the hindrance of the second fixed point.

All these methods involve the tacit assumption that each lever is moved only by the contraction of its parallel muscle. This assumption, however, is quite gratuitous, and we soon came to realize that it is not valid; for if a piece of solid material, say a rubber band, is pulled so as to make it longer in one direction, it must necessarily become narrower in the other direction. How this works in the case of the intestinal wall is shown by the dotted lines of figure 1. These were sketched from pieces of intestine, whose spontaneous movements had been abolished by cooling to room temperature. They were pinned on a moist sheet of smooth paper, to minimize friction, and then slightly stretched, first in one direction, then allowed to resume their original shape, and then stretched in the other direction. It is quite evident that every movement in one direction, say a contraction of the longitudinal muscle, would cause a change in the other direction, in this case a widening simulating a relaxation of the circular muscle! Figure 7 illustrates by an actual experiment the deception that results. This tracing was taken from a triangle of active intestine arranged as in figure 1, *III*, suspended in a warmed aerated Locke solution. As seen at the left, the tracing showed beautifully symmetrical pendulum contractions in both the longitudinal and circular limb. But when the hypothenuse of the triangle was incised in the direction of the dotted arrow, thereby diminishing the distortion of the muscles by each other, the contractions in the circular direction (lower tracing) became much weaker; and when the incision was carried deeper (but with a bridge of tissue at the right angle still intact) the circular "contractions" disappeared entirely. Very evidently the circular muscle had not contracted at all, but had been passively pushed and pulled by the contractions of the longitudinal muscle. Simultaneous tracings from such preparations are therefore contaminated and may be obscured or entirely simulated by artefacts. This holds true equally for intact intestine. True records of the movements of the two muscles can be obtained only by cutting the longitudinal or the circular strips so narrow that distortion by the other muscle becomes negligible. Figure 7 showed that this may be done simply by deeply bisecting the right-

angled triangle, as indicated by the dotted arrow in figure 1, *III;* but it is more advantageous to cut away the muscles in the center of the triangle, since they exert little pull on the levers that are attached near the margin, but must somewhat complicate the mechanical conditions. The margin of the triangle which is thus left represents an L-shaped segment, with one limb in the direction of the longitudinal, and the other limb in that of the circular fibers (fig. 2, *III*). The angle preserves a physiological connection through which nervous and muscular conduction could take place; and since the two limbs are taken from the same segment of the intestine, they present the best condition for segmental correlation. In such preparations the two limbs contract without any perceptible interference, or if only one contracts, the other lever traces a straight line.

This is the principle of the method that was used in the following studies. Its successful application requires the careful observance of certain precautions, which we outlined briefly in an earlier paper (Siaulis and Sollmann, 1926), but which require more detailed discussion. The more important of these precautions concerns the avoidance of unnecessary handling, of drying, and especially of asphyxia. The latter requires not only continuous aeration, but also the use of thin strips of tissue, and the removal of the mucous membrane, either by clipping it away with scissors, or less effectively by autolyzing under refrigeration. This is particularly important for the circular muscle. In L-shaped preparations made from fresh intestines with intact mucosa, the longitudinal limb generally started promptly with vigorous rhythmic contractions and without serious change of tone; but the circular limb usually does not develop a rhythm or ceases in a short time, and generally shows marked tone changes, namely, some primary relaxation followed by a secondary high rise of tone. In clipped fresh preparations, on the other hand, the circular limb generally behaves similarly to the longitudinal, rhythmic contractions setting in promptly, and becoming more and more vigorous, with little change of tone.

The special importance of the removal of the mucosa from the circular limb is due to the relative inaccessibility of the inner muscle-coat to the solution, and its consequent greater liability to oxygen deficiency and to the accumulation of injurious metabolic products: Its outer surface is covered by the sheet of longitudinal muscle; and what is even more serious, the circular limb rolls up transversely into a tight quill with the mucous surface outward, as shown photographically in figure 4, so that practically only the thick mucosa is exposed in the solution. It may be seen from figure 4 that the longitudinal limb does not show this quilling. It would tend to roll in the longitudinal direction but this is prevented by the weight of the lever. L-shaped preparations cut from intestines that have been kept on ice for a day or longer also develop the quilling gradually, after their muscle becomes active in the warmer solution, but in their case the



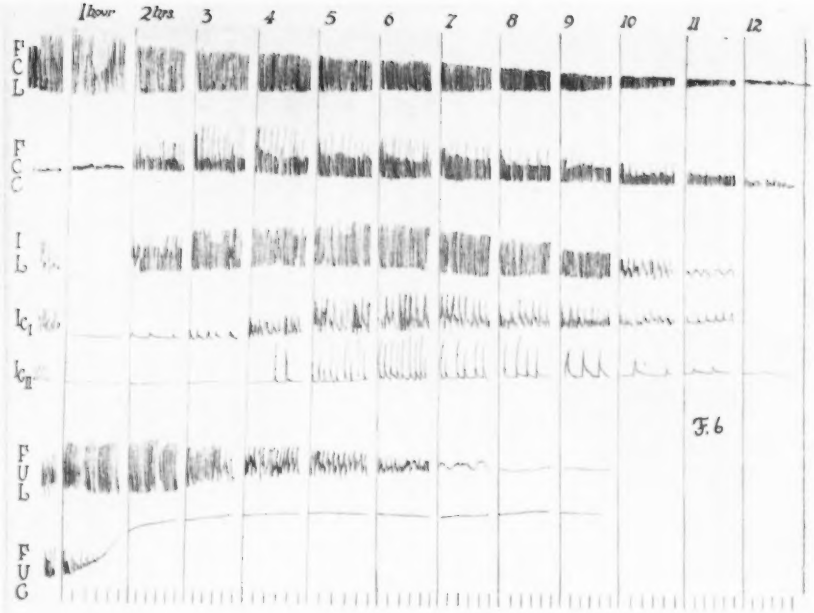


Fig. 4. The rolling-up of the L preparation: the circular limb is "quilled," with the mucous surface outward, precluding the access of oxygen. The longitudinal limb does not undergo the quilling.

Fig. 5. Support for the L-shaped segment and levers.

Fig. 6. Course of contractions in the different types of preparations. This is represented diagrammatically for five minutes at the beginning of each hour. The upper and second represent the longitudinal and circular limbs of clipped fresh intestine; the third, the longitudinal limb, and the fourth and fifth, the two types of movements of the circular limb of iced intestine; and the sixth and seventh the longitudinal and circular limb of the unclipped fresh intestines.

mucosa was found to cause less interference with the circular muscle so so that even unclipped preparations usually become rhythmic. It was also noted that the mucosa of the iced intestine had a different consistence and tends to detach shreds when immersed in the Locke solution. The microscopic examination of fixed and stained sections of such intestines, which were kindly prepared by Miss E. F. McCallum of the Pathology Department, showed that during the icing the mucous membrane has undergone extensive degenerative changes, presumably autolytic, and has thereby become much thinner, much looser, and presumably much more permeable. The immersion of either fresh or new preparations in warmed aerated Locke solution for three to five hours respectively does not produce further degeneration.

TECHNIC OF L-SHAPED SEGMENTS. The lower part of the ileum of rabbits was used in this series of experiments. It is advisable to practice and adhere to a uniform technic so as to save time and thereby to minimize exposure and asphyxia. The animal is killed by a blow on the neck, the peritoneum is opened, and a piece of the intestine about 8 cm. in length is gently removed, rinsed cautiously inside and out with a stream of warmed Locke solution, and then placed immediately into aerated Locke solution of 38°C. (gram per liter: NaCl, 9.0; KCl, 0.42; $CaCl_2 \cdot 2H_2O$, 0.24; $NaHCO_3$, 0.3; glucose, 1.0). Fresh preparations are used at once. The intestine is lifted on the forefinger, the site of maximal relaxation is selected, and from this a segment 1 to 1.5 cm. long is quickly cut with sharp scissors. When several preparations are to be compared, care is used to make them of uniform length. The segment is then cut open with the scissors, parallel and close to the mesenteric insertion (fig. 2, *I*), returned to the aerated, warmed solution in a Petri dish, and there spread out flat, mucosa upward, on a piece of paraffined cork. The four corners are slightly stretched and fastened with pins as shown by solid lines in (fig. 2, *II*). The transverse diameter should be about a third to a half longer than the longitudinal, to compensate for the relative feebleness of the circular coat in this region.

If the mucosa is to be clipped, and this is the standard procedure, this is performed with scissors, curved rather sharply on the flat; denuding a strip about 3 mm. wide on the margin opposite the mesentery, and a similar strip at right angles to this, as shown in the shaded area of figure 2, *II*. It is desirable to cut rather deeply, but without injuring the muscle. The clipping is done under the warmed aerated Locke solution, in the Petri dish, occasionally passing a few bubbles of air between the serosa and the cork by means of a fine pointed pipette, to prevent asphyxiation.

When the mucosa has been removed (or directly in "unclipped" preparations), a second set of pins is inserted as shown by the dotted pins in figure 2, *II*, and the intestine is cut with a sharp safety-razor blade, as shown in the dotted lines of figure 2, *II*, giving an L-shaped segment about

2 mm. wide (fig. 2, *III*). This is then attached to the recording device, (fig. 2, *IV* and figs. 5 and 3). The angle is attached to the hollow supporting rod by piercing the tissue with the sharp point of the small hook. The ends of the limbs *C* and *F* are pinched with delicate wire serrefines, and connected by threads with the respective levers. The moist serrefines are rather slippery and should be handled with forceps rather than with the fingers. The circular limb can be easily identified by the attachment of the mesentery (fig. 2, *III*).

For making *iced preparations*, pieces of intestine of 8 cm. length are removed from the freshly killed animal, rinsed inside and out with warmed Locke solution and are then dropped into a beaker of Locke solution, that has been kept in the refrigerator, at 0 to 3°C. and which is aerated for a few minutes immediately before receiving the intestines. It is replaced at once in the refrigerator for 24 to 36 hours. The preparation is then made as with the fresh intestine, but the manipulations are carried out in refrigerated Locke solution. This reduces the risk of handling and of asphyxia, and the immobility of the cold intestine greatly facilitates the operation. As has been said, iced intestines generally give good contractions without clipping, but clipping would doubtless be advantageous. However, the iced preparations of our experiments were all unclipped; in some cases the shredded mucosa was thinned by brushing. As will be explained later, the iced preparations do not reach their full activity until they have been in the warmed solution for some time.

The recording apparatus were of the type customary for the study of excised intestines. Figure 5 shows the arrangement of the levers. These had a magnification of 7 for the longitudinal, and of 10 for the circular limb. They were weighted to have an overload of 0.5 gram, adjusted by suspending a rider from the lever at a point (ascertained and marked once for all) where it balanced a 0.5 gram weight suspended from the serrefine in place of the muscle. The volume of the intestinal limb is so small that the buoyant effect of the immersion in Locke solution was neglected.

Figure 3 shows the bath for the Locke solution. This had the capacity of 200 cc. and was provided with inflow and overflow tubes, so that the solution could be changed practically without disturbing the preparation. As two experiments were always run parallel, two baths and two sets of levers were arranged in a glass-tank filled with warm water. The temperature was maintained within 0.1° of 38° by an electric thermo-regulator. Compressed air was used for aeration. This was admitted through the cannulated support, with a rather narrow opening. The latter necessitates a higher pressure, but the smaller bubbles avoid mechanical disturbance of the preparations and threads. Because of their larger surface the small bubbles also insure better saturation.

COURSE OF THE INTESTINAL CONTRACTION UNDER STANDARD CONDITIONS. The movements of our intestinal segments present the same types as the familiar contractions of the Magnus segments. They include the regular

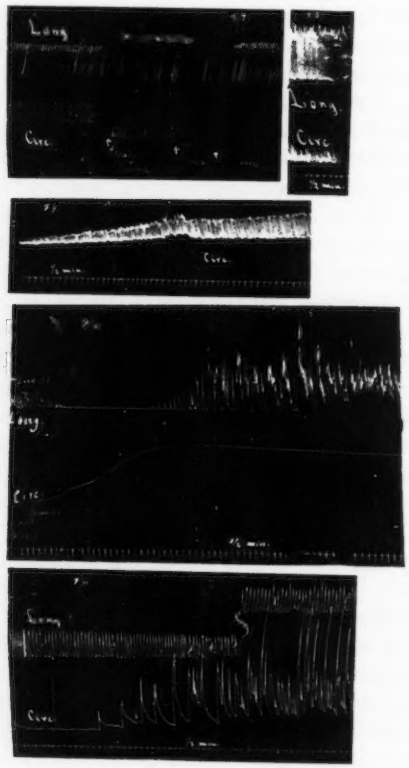


Fig. 7. Effect of incising the triangle as arranged in figure 1, III (about three times the actual size).

Fig. 8. Initiation of contractions in fresh clipped intestine. Vigorous pendulum movements start immediately, and continue for hours. (The time markings in all the tracings represent half minutes.)

Fig. 9. Progressive improvement of pendulum contractions. Circular limb, starting with immersion.

Fig. 10. Iced intestine. Upper tracing: Initiation of rhythm in iced intestine (longitudinal): Immediate contraction, pause, twitches, peristaltic waves, peristaltic spasm. Lower tracing: Tonic rigor of circular (unclipped) limb.

Fig. 11. Delayed initiation of contractions in iced intestine (50 hours in the refrigerator). The preparation has been in the warm aerated Locke solution for two hours, and the circular limb is just starting to contract. The longitudinal limb had been running these rapid peristaltic contractions for a long time.

relatively rapid pendulum movements, the slower rhythmic waves corresponding to the peristaltic contractions and which for brevity we may call by this name; a somewhat abnormal type of rhythmic twitches; a more persistent tonus change with "tonic spasm," and "tonic rigor," i.e., permanent rise of tone associated with loss of irritability. The significance of these types will be discussed later. The movements of the longitudinal and of the circular limbs, and of fresh and iced preparations agree in their general features, although the details vary with conditions; and as conditions are never quite identical, the course of the contractions is never quite the same for different intestines, nor for the two limbs of the same preparation. When the experimental conditions are standardized, as has been described, the differences depend mainly on the preliminary treatment of the intestines. Consequently, the course of the contractions can be grouped into several types that are characteristic for the longitudinal and circular limbs of the fresh clipped, the iced and fresh unclipped preparations respectively. These are illustrated diagrammatically in figure 6.

In fresh clipped preparations, the longitudinal and circular limbs behave very similarly. Their contractions start immediately after immersion (fig. 8) and continue ten hours or longer. They are generally a very regular pendulum rhythm, with little tendency to peristaltic waves. At first they are often somewhat irregular and somewhat weak in the circular limb and improve progressively (fig. 9) reaching their optimal inside of an hour. Critical experiments should therefore be deferred to this time. After three or four hours the amplitude of the contractions declines progressively, but very gradually. After nine or ten hours, the contractions of the circular limb are often interrupted by short pauses.

Iced preparations (fig. 6, third to fifth), on being transferred to the warmed bath often make a few vigorous contractions, due presumably to the stimulation of the sudden change of temperature. These endure only for a very few minutes and are succeeded by absolute quiescence, a "dormant period." This occurs in all iced preparations and never in fresh preparations; it is therefore evidently an after-effect of the icing. This suspension of the contractions lasts ten to thirty minutes for the longitudinal limb and much longer, from one to four hours, for the circular limb.

After the dormant period, the longitudinal limb and the majority of the circular limbs (about two out of three) begin to contract, first with single twitches, then with interpolated contractions, constituting the peristaltic type (fig. 10, longitudinal; fig. 11, circular). These peristaltic contractions continue, often with great regularity (fig. 12, longitudinal) to about the eleventh hour, i.e., about as long as in the fresh clipped preparations. They decline progressively and very gradually, much as the pendulum movement of the fresh clipped preparations (fig. 6, third and fourth). The

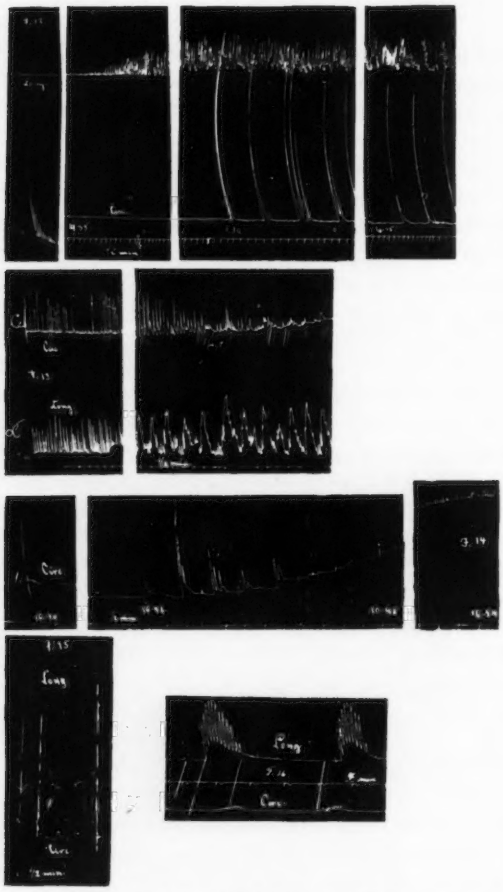


Fig. 12. Twitch contractions of the circular limb. These occur in about a third of the iced preparations. This tracing also illustrates the dormant period of the iced intestines. The preparation had been refrigerated for thirty hours. The first tracing starts immediately on immersion; the second twenty minutes; the third one and one-quarter, the fourth two hours after immersion. The rhythmic twitches continued several hours practically unchanged, until the experiment was terminated.

Fig. 13. Development of tonic rigor in the circular and development of peristalsis in the longitudinal limb (fresh unclipped intestines).

Fig. 14. Tonic rigor of the circular limb, preceded by fall of tone, and starting with a few strong twitches, the first twelve minutes after immersion. The third tracing is twenty-four minutes after immersion.

Fig. 15. Non-correlation of the longitudinal and circular muscle. The straight lines connect corresponding points of the two tracings. These were taken at a faster speed, from fresh unclipped ileum.

Fig. 16. Non-correlation in the haustra of the ascending colon. The peristaltic wave of the longitudinal limb starts first in the left tracing, and last in the right tracing (fresh unclipped preparation).

best time for critical experiments would be between one and three hours after the initiation of the contractions.

In about a third of the circular limbs the contractions after the dormant period start suddenly, and continue, as maximal periodic twitches separated by pauses (fig. 12). After the seventh hour the pauses become gradually longer and the contractions weaker (fig. 6, fifth).

The excitable intestines generally do not undergo significant changes of tone. Occasionally the circular limb goes into tonic rigor (fig. 10, longitudinal).

Fresh unclipped preparations (fig. 6, sixth and seventh) usually start to contract at once on immersion, with rapid somewhat irregular twitches. The *longitudinal limb* generally progresses promptly to regular peristaltic contractions (fig. 13) which decline gradually, but more rapidly than in the unclipped or iced preparations, so that the movements practically cease in six or seven hours. *Fresh unclipped circular limbs*, if they are prepared with such rapidity that they are suspended before the rolling up or quilling has begun, may start with vigorous pendulum movements, just as the unclipped. Soon, however, the contractions become slower and progressively weaker and the tone rises progressively as in figure 13. If operations are not performed so rapidly, and the limb has begun to roll before it is suspended, the rhythmic contractions fail entirely to appear, but instead there is a progressive and considerable rise of tone, similar to that shown in figure 10. When this has occurred, the preparations never return to rhythm. It is therefore apparently a sort of tonic rigor, induced by the asphyxiation consequent on the quilling of the preparations. In intermediate cases, there may be a few irregular twitches before the tone rises (fig. 14).

Correlation of the longitudinal and circular contractions. In normal peristalsis, as observed for instance on perfused excised intestine, a correlation appears to exist in the movements of the two sheets of muscle. The L-shaped segments should help to answer the question whether this correlation is effected by nervous or muscular conduction, or whether it is due merely to the coincidence of the mechanical stimulus of the distention; for the latter is absent in the L-shaped segments whilst the possibility of muscular and nervous coördination is preserved by the continuity at the angle of the L. It was observed that notwithstanding this connection, the movements of the two limbs are often quite dissimilar, so that one limb may show only pendulum contractions, the other strong peristaltic waves. When waves are present in both limbs, their type and rate is often quite different (figs. 11, 12, 13). In the faster tracing of figure 15 the corresponding points have been marked by electric signals. Even when the peristaltic waves are similar, there is no uniformity in the details or in the sequence of individual contractions of the two limbs. This may be seen

from the corresponding points in figure 16, from the haustra of the ascending colon (fresh, unclipped), where the wave starts sometimes first in the longitudinal limb (at left of the figure), and sometimes first in the circular limb (right of the figure). It appears from this that the coördination of the longitudinal and circular contractions of the intestines is not effected by nervous or muscular conduction, but presumably by the distention. However, the evidence is not quite conclusive; in the first place, it is not possible to insure perfectly equal oxygenation of both limbs, and their excitability may therefore be unequal. It might also be objected that the bridge of tissue may not be sufficient for normal conduction, but this appears improbable, in the light of experience with other conducting structures, such as in the heart.

LOAD ON INITIATION OF CONTRACTIONS. Before employing our preparations for pharmacologic studies, it appeared advisable to investigate the controllable physiologic conditions, so as to adjust these to the optimal, and to recognize, regulate and utilize departures from the normal. Among the more important of these physiological variables are the load, aeration and temperature, which we shall describe in this paper. These may be considered from the two distinct aspects of their influence on the initiation of contractions, and on established contractions. We may begin with the effect of load on the initiation of contractions. This was studied on iced preparations, which alone show a definite dormant period. The load was calibrated by actual weights suspended in place of the muscle. It may be remarked that the application of equal weights to different preparations does not really mean equal tensions on the muscle; for the preparations cannot be made of absolutely uniform size and shape, and the weight is therefore distributed over different amounts of membrane and fibrous tissue. In the unclipped preparations a part of the weight is also borne by the mucosa. Great accuracy is therefore out of the question. The tension to which the muscle is subjected may be judged somewhat by the rise or fall of the lever during the first twenty or thirty minutes. The weight of 0.5 gram produced practically no change in the tone, either in the circular or longitudinal limbs. This weight of 0.5 gram also appeared optimal for the development of the contractions, whilst heavier and lighter weights appeared to be unfavorable. The *circular* limb was distinctly more sensitive, both to heavy and to light loads, and either failed to contract at all, or contracted very weakly, with the heavier weights (1.5 grams) and with the lighter weights (0.1 to 0.3 gram). The *longitudinal* limb is more resistant: contractions appeared even with weights as heavy as 3 grams, although they were rather weak. With light weights (0.1 to 0.3 gram) the contractions start vigorously, but often develop rise of tone with decreasing excursions.

THE EFFECT OF LOAD ON THE LENGTH OF CONTRACTING INTESTINE. This

was studied after the contractions had been going for an hour, by increasing the original weight by a definite percentage. The descent of the lever was measured from the midpoint of the contractions. In a given preparation, the extension increases with the weight, but different preparations are stretched to a varying degree by the same weight, and are therefore not comparable. The form of the curve, however, is fairly uniform for different weights and preparations, for circular as well as longitudinal. There are, however, some characteristic differences between the fresh clipped and the iced unclipped preparations. Both have the asymptotic form of curve described by Evans, a constant length being reached only

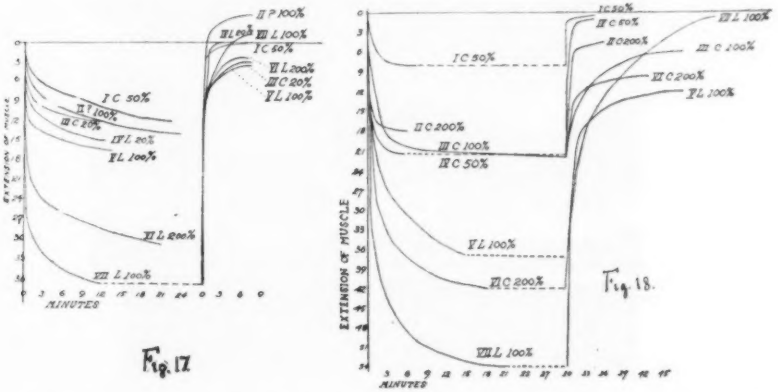


Fig. 17. Chart of the effect of varying loads on the clipped fresh intestines. The chart begins with the effect of increasing the normal load (generally 0.5 gram), plotted against the time during which the extra weight was applied. Broken lines are carried horizontally from the time when the extra weight was removed, to the right half of the chart, which shows the recovery of the length of the muscles under the normal load.

Fig. 18. Chart of the effect of load on unclipped iced intestine.

slowly. This is more marked with the fresh clipped preparations (fig. 17). The unclipped iced (fig. 18) reach their maximal extension quite promptly, especially with light loads. On removing the load the behavior of the two preparations is reversed: The fresh clipped (fig. 17) preparations return practically to their original level very promptly, regardless of the degree of stretching that they had undergone. The iced preparations (fig. 18) return more slowly and uniformly; the lag being the greater, the more they had been extended by the load. The very prompt return of the length of the fresh clipped preparations throws doubt on Evans' suggestion that after-extension is due to the viscosity of the cytoplasm, for this should operate equally to delay the return on unloading. We are not prepared

to say whether the differences between the fresh clipped and the iced unclipped preparations are due to changes in the protoplasm produced by icing, or to the presence in the iced preparations of the mucous membrane, which undoubtedly has its own extension curve.

THE EFFECT OF LOADING ON THE PENDULUM MOVEMENTS. Both in the longitudinal and in the circular limbs, increase of load decreases the amplitude of the pendulum movements proportionally to the load; and these return to the normal as the weight is decreased. This is best seen in the fresh clipped preparations (fig. 19).

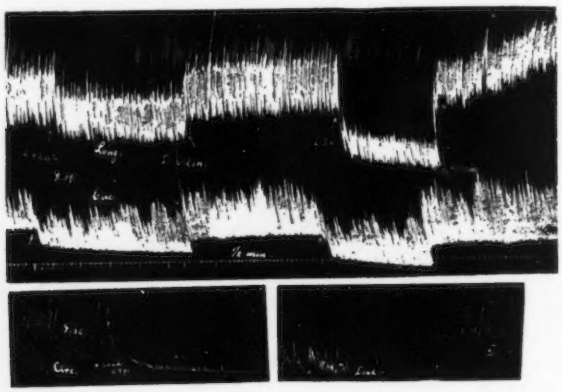


Fig. 19. Effects of load on length and on pendulum movements. Fresh clipped preparations. The tracing starts with the usual load of 0.5 gram. At the first arrow, this is increased by 20 per cent. At the second arrow, it is restored by 0.5 gram. At the third arrow it is increased by 100 per cent; and at the last arrow it is restored by 0.5 gram. The amplitude of the pendulum movements varies inversely to the load.

Fig. 20. Effect of the load on the peristaltic waves. In the left tracing, doubling the normal load of 0.5 gram converted the high and slow waves of the circular limb of the iced intestine into very shallow and very rapid waves. Conversely, unloading in the right tracing, makes the peristaltic waves higher and slower.

THE EFFECTS OF LOADING ON THE PERISTALTIC MOVEMENTS. These are best seen in the iced preparations in which peristalsis is ordinarily most pronounced. In these, increase of load diminishes the peristaltic excursions very markedly and increases the rate; whilst decrease of load increases the excursions and diminishes the rate. In other words, the rate tends to go parallel to the load and the excursions, inversely. Figure 20 shows these phenomena on a circular limb; The longitudinal takes exactly the same course. The effects of loading on fresh preparations, clipped and unclipped, are also in the same direction, but less marked since fresh preparations do not ordinarily show typical peristaltic movements.

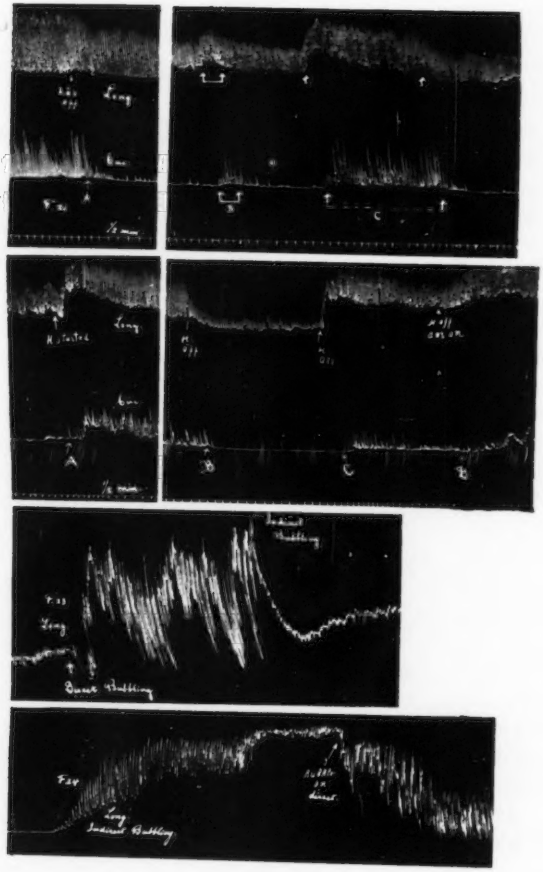


Fig. 21. Effect of agitation on asphyxiating intestine. The clipped fresh intestine was being aerated with indirect bubbling. At the first arrow the aeration is stopped and the pendulum movements at once become more shallow. The second tracing starts when the aeration had been stopped for twenty-two minutes. The surface of the liquid was lightly tapped between the first pair of arrows, and gently stirred between the second pair. The response was immediate, and especially large in the circular limb.

Fig. 22. Aeration with hydrogen and with air. This is a continuation of tracing 21. The aeration had been stopped for nearly half an hour, when a stream of hydrogen bubbles was started at *A*. Considerable improvement resulted. After sixteen minutes, the hydrogen was stopped, at *B*, and the depression recurred at once, and to a greater degree than before the hydrogen. At *C* the hydrogen was again started, and again produced improvement, but less than at A. At *D* the hydrogen was replaced by air, with marked further improvement. There is a six-minute gap between the tracings.

Fig. 23. Contrast of direct and indirect bubbling on unclipped fresh intestines. The tracing begins with the air bubbles directed away from the muscle. At the first arrow they are directed against the muscle; and at the second arrow, they are again directed away.

Fig. 24. Direct bubbling relaxing intestinal spasm. The spasm occurred spontaneously, presumably by partial asphyxia, in the unclipped longitudinal limb of an "iced" intestine.

THE EFFECTS OF AERATION AND AGITATION. The movements of excised mammalian smooth muscle require continuous aeration of the solutions in which they are suspended. This would naturally be attributed to the need of oxygen, and in the end, a supply of oxygen is of course indispensable, especially for tone. However, Evans and Underhill (1923) showed that aeration with indifferent gases (nitrogen, hydrogen) produces effects at least temporarily similar to those of oxygen aeration. Tapping the vessel or touching the muscle with a soft brush also produced analogous effects, the direction of the response varying with the organs and conditions. These observations were confirmed by Simonart (1926). Our preparations also gave these responses. For instance, in figure 21, with fresh clipped intestine, stoppage of air current at *A* diminished the excursions of the longitudinal and especially of the circular limb; the circular being generally more sensitive to this as to other disturbances. At *B*, twenty-two minutes later, the weakening had progressed considerably. At this point the surface of the solution was lightly tapped: both segments show immediate stimulation by increase of tone and excursions. At *C*, the solution was gently stirred, without touching the muscle: this resulted in more marked stimulation. When the stirring was stopped, the muscle was again weakened. Figure 22 represents the same intestine which had then been without aeration for over one-half hour. At *A*, a current of hydrogen was started. This produced immediate stimulation, very similar to the stirring and doubtless due to the same cause. At *B* the hydrogen was stopped: the contractions weakened greatly, the circular movements being entirely suppressed. At *C*, the hydrogen was started and the muscle improved at once. At *D* a current of air was substituted for the hydrogen; this produced very marked and progressive improvement.

With ordinary aeration a great difference was found to exist according to whether the air bubbles were delivered at a little distance from the muscle, or whether they were made to impinge directly on the muscle as they do when the air is delivered as usual through the L-shaped support. When studying these differences, the air was delivered through a separate fine pointed tube, bent so that a stream of fine bubbles could be directed either against the muscle (direct bubbling) or along the wall of the bath, 2.5 cm. removed from the muscle (indirect bubbling). The air and oxygen supply and the number of bubbles, and therefore the amount of agitation of the solution, were the same in both cases. The effects are illustrated in figure 23 on the longitudinal limb of a fresh unclipped intestine. The tracing began with indirect bubbling. When the bubbles were directed against the muscle, there resulted an immediate large rise of tone and excursions. When the bubbles were again directed away from the muscle there was immediate decrease of tone and excursions. On the other hand, direct bubbling may release tonic spasm as in figure 24, an iced

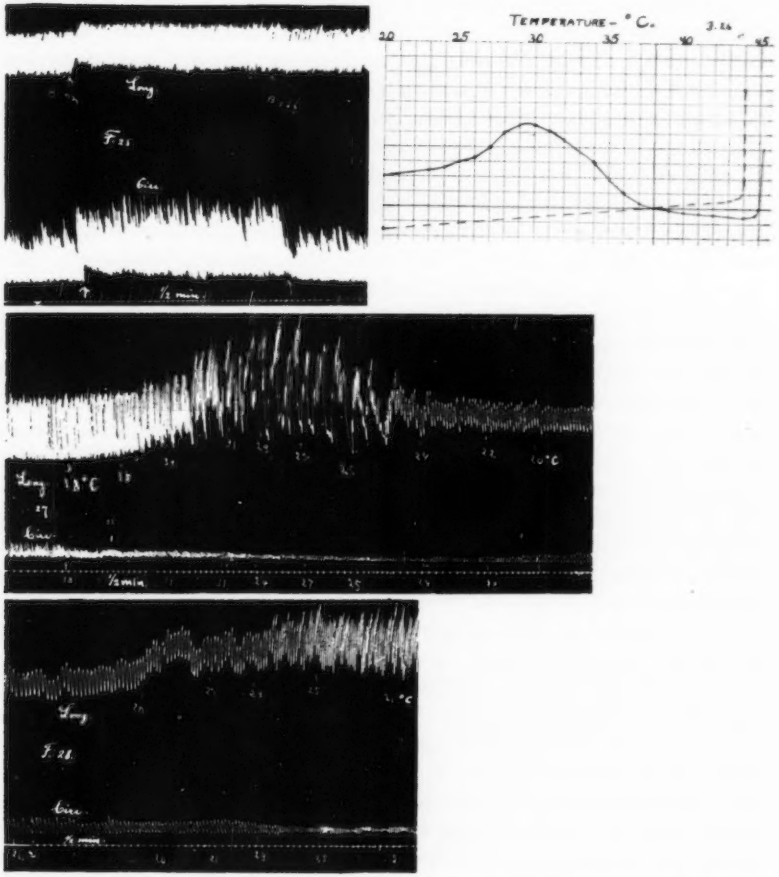


Fig. 25. The improvement by direct bubbling is relatively small in clipped fresh intestine, presumably because the clipping secures an adequate oxygen supply for the thin strip of muscle.

Fig. 26. Diagrammatic curve of tone changes with gradual alterations of temperature. The curve is drawn diagrammatically by combining the diastolic tone of a number of typical tracings. The solid line represents the tone of the longitudinal, the broken line that of the circular limb.

Fig. 27. Effects of cooling from 38 to 20°C., clipped fresh intestine. Note the fastigium of tone and peristalsis in the longitudinal limb, at 29°. The pendulum rate in both limbs varies inversely to temperature.

Fig. 28. Warming from 20 to 27°C. The tracing is a continuation of figure 27. The preceding cooling has rendered the intestine somewhat sluggish, and the peristaltic waves are consequently less high than in figure 27.

longitudinal limb. The stimulative effects of direct bubbling are most marked in the fresh unclipped intestine, next in the iced. They are relatively small in the fresh clipped preparations (fig. 25). This suggests that the effect of the agent is not due to mechanical stimulation of the muscle, as Simonart supposed, for there is no reason why mechanical stimulation should act differently on clipped and unclipped preparations. On the other hand the difference is understandable on the basis that the access of oxygen and the removal of metabolites (as suggested by Evans and Underhill) is proportional to the renewal of the solution in contact with the muscle by agitation, as contrasted with the slowness of diffusion. This agitation is less important for the clipped intestine because this is in effective contact with the solution on both sides whilst the unclipped preparations are practically impervious on the side of the mucosa. The stream of hydrogen also acts merely by agitating the solution which still contains some dissolved oxygen. Comparison of *A* and *C* in figure 22 shows that the effect of hydrogen agitation decreases rapidly as the oxygen is used up; while direct bubbling with air causes marked improvement. Direct mechanical stimulation of the muscle by the bubbles is also discredited by the fact that there is practically no stimulation on rhythmically tapping the stand on which the muscle is supported, a procedure which should furnish mechanical stimulation of the muscle with a minimum of agitation of fluid.

It may therefore be concluded that agitation and especially "direct bubbling" stimulates smooth muscle by dislodging the stagnant fluid in contact with the muscle and thereby favoring the access of fresh fluid and especially of dissolved oxygen.

Response to gradual changes of temperature. These were studied after leaving the preparations in the aerated Locke solution at 38°C. until the tracings showed uniform contractions; then adding gradually and at regular intervals small portions of iced or warm water to the tank so as to change the temperature of the saline bath at the rate of about 1°C. per minute. Generally the bath was first cooled to a varying extent, sometimes to 20°, then warmed again, to 46°. The various types of preparations, fresh, clipped and unclipped and iced, behave alike; but there are marked differences between the longitudinal and circular limbs, and both react quite differently from the schema that Evans gives for the response of smooth muscle tone to gradual changes of temperature (page 375 of his review of the physiology of plain muscle). According to this schematic description, the tone of smooth muscle relaxes with rise of temperature, and increases with fall of temperature. At 45° to 48°C. the fully relaxed muscle becomes paralyzed, but may be revived by cooling; at 50°C it is speedily killed, that is, it is not revivable, but is still relaxed.

As has been said, our results do not fit into this schema. Figure 26

shows the typical course of the "diastolic tone" in our experiments. It was drawn somewhat diagrammatically from the plotted measurements of six typical experiments, selected from a total of sixteen experiments on fresh clipped, six experiments on iced and two on fresh unclipped intestines. All had essentially the same form, although the changes were not of the same extent, and the rise and fall of tone occurred more or less steeply, partly according to the excitability and partly with the speed of the temperature changes. The form of curve was also the same whether plotted in the direction of the descending or of the ascending temperature; the details were not quite identical, because prolonged cooling renders the muscle somewhat sluggish in responding to heat.

It will be seen from this figure that the *tone of the circular* limb increases slightly with the temperature (i.e., the contrary of Evans' rule), between 20° and 43°; between 43° and 45° there is an abrupt rise of tone with smaller and irregular excursions, that is, a tonic spasm. The tone of the longitudinal limb undergoes greater and more complex changes. Between 30 and 40° it follows Evans' rule, relaxing with heat and rising with cold, i.e., its course is the opposite of that of the smooth muscle; but from 30° down, it relaxes with cold. There is thus a critical temperature from which the tone of the longitudinal muscle slopes downward in both directions, as illustrated in figures 26 and 27. This is a region rather than a point, shifting somewhat in different experiments between 27° and 36°. Above 45° the longitudinal muscle goes abruptly into tonic spasm. In brief, the changes in the longitudinal tone are similar to those in the circular tone at the extremes of temperature, below 27° or 30°, and above 42° or 45°. In the intermediate range, between 30° and 40°, the tone of the two muscles runs a diverging course.

The departures of these reactions from the simple schema of Evans are somewhat connected with *the effects of temperature on the rhythmic movements*, which doubtless interact with the tone. These are also rather complex, but again quite uniform in type for the different preparations. They are shown typically in figures 27, 28 and 29 which reproduce continuous tracings from an experiment on fresh clipped intestine. The phenomena are again most simple for the *circular preparations:* cooling (fig. 27) produces progressive slowing of pendulum movements, the excursions being also weakened to 24°; cooling beyond this somewhat increased the excursions, presumably as a result of the slowing. Warming (figs. 28 and 29) causes progressive increase of the pendulum rate. The excursions are first somewhat decreased, to 25°; but above this they increase progressively, and above 38° they develop peristaltic grouping. Above 43° they go abruptly into the tonic spasm with irregular and somewhat slow rhythm.

In the longitudinal limbs, as in the circular, the rate of the pendulum

movements varies strictly and the amplitude generally in the direction of the temperature. Excessive heat also causes abrupt tonic spasm at a temperature perhaps a degree higher than with the circular limb. The longitudinal differs from the circular in the tone changes between 25° and 44° as has been described; and in the production of peristaltic waves.

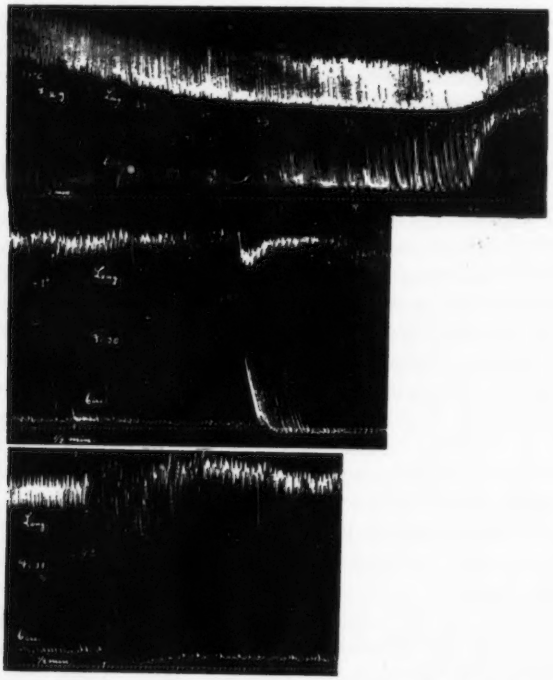


Fig. 29. Warming from 27 to 45.5°C. Continuation of figure 28. Note the differences in the behavior of the longitudinal and circular limbs between 29 and 43°; and the tonic spasm of both limbs at 45°.

Fig. 30. Abrupt heating (clipped fresh intestines).

Fig. 31. Abrupt cooling (this tracing was taken just before figure 30, from the same preparation).

These appear in the longitudinal limb on cooling, after the tone has arisen to a certain level, in this experiment about 33°, and increases with further cooling, in proportion to the rise of tone, the two reaching their maximum at the same temperature, in this experiment about 28°. With further cooling, the tone and peristaltic waves again decrease together and disappear, in this experiment about 24°. On raising the temperature the waves

tend to recur generally less marked than on cooling and not always at exactly the same point, due to the persistence of the cold-inertia. The rate of the peristaltic waves varies directly and uniformly with the temperature. The peristaltic excursions as has been explained reach their maximum at the tone fastigium, i.e., generally between 27° and 36°. The peristaltic activity and the tone therefore go together. The tone changes are evidently not caused by peristalsis for they may occur with slight peristaltic activity as in figures 28 and 29. Either, therefore, peristalsis and tone are both expressions of increased irritability; or the peristalsis is the result of increased tone.

If we attempt to generalize we may say that only one temperature phenomenon is uniform, namely, that the pendulum movements are slowed by cold and quickened by heat throughout the whole range of the observed temperature. The amplitude of the pendulum movements is also diminished by cold and increased by heat, but there are some complications at the extremes of temperature.

The tone reacts differently in the circular and longitudinal limb: In the circular limb the phenomena are simple, consisting in a slight rise of diastolic tone for the whole range of temperature between 20° and 43°. The tone of the longitudinal limb runs a more complex course with a maximum at 27° to 36°, and relaxation both above and below this temperature.

Peristaltic waves appear and reach their maximum with increase of tone, in the longitudinal about 27° to 36°, in the circular about 38°. Above 43° both limbs go into tonic spasm, instead of relaxing according to Evans' schema.

ABRUPT CHANGES OF TEMPERATURE. These act as immediate direct stimuli (Evans) so that the mechanism of the immediate response contains elements which are not present in gradual alterations of temperature. In our experiments abrupt changes of temperature of the saline bath, from 0.5° to 10°, were produced by adding iced or hot Locke solution directly to the bath through a long stemmed funnel, so that the added fluid would mix with the bath before reaching the muscle. The temperature was then allowed to return gradually to 38°. Nearly one hundred experiments were made with all kinds of preparations. The results were practically identical, but the responses of the longitudinal and circular limbs differ materially in sensitiveness and in degree and also in kind and in direction. The effects are distinct with 0.5° in the circular and generally with 2° to 4° in the longitudinal. They increase with the magnitude of the temperature changes.

The effects of abrupt heating are illustrated in figure 30 from a fresh clipped preparation. Peristalsis is abolished in both limbs whilst the pendulum movements are increased in rate and in excursions. The increased amplitude is especially marked in the longitudinal limb and in this is soon

followed by exhaustion, which may lead to complete suspension of visible contractions.

The tone appears to go in contrary direction in the two limbs. The longitudinal shows immediate relaxation, whilst in the circular the relaxation is preceded by rise of tone. The difference may be merely apparent, and due to incomplete relaxation between the high and rapid pendulum contractions of the circular coat.

The effects of abrupt cooling are shown on the same intestine in figure 31. The pendulum movements are abolished in both limbs, and both show a temporary fall of tone; otherwise they appear to take a divergent course. In the circular limb, all movement is suspended for several minutes, generally much more completely than in figure 31. With cooling by 10°, the suspension may last half an hour. With the longitudinal limb, the fall of tone is accompanied by much higher but somewhat slower peristaltic waves, and is followed by rise of tone.

Comparing the effects of abrupt heating and cooling on the two limbs it may be seen that the tone in all cases tends to be lowered at some stage; but this is preceded by a slight rise when heating the circular limb and is followed by a considerable rise when cooling the longitudinal. The peristaltic excursions are abolished by heat and tend to be increased by cooling. The pendulum movements are increased in height and rate by heat, and abolished by cold.

TYPES OF INTESTINAL MOVEMENTS. Thus far, we have attempted to describe the behavior of the two muscle-sheets of the intestines under a variety of physiologic conditions. This behavior includes so many concurrent phenomena that it is difficult to focus them into a unified picture and to grasp their significance. It is therefore advisable to recapitulate the subject from the standpoint of the varieties of intestinal movements. As exhibited by the thin longitudinal and circular strips, these may be grouped under six fairly well defined types:

1. Pendulum movements. These are the uniform contractions, of very regular and relatively rapid rhythm, approximately fourteen to sixteen per minute, about the same in both limbs (fig. 19). Their rate increases and diminishes with the temperature (figs. 28 and 29), but it is little affected by other conditions, such as general excitability (fig. 9), load (fig. 19) or asphyxia (fig. 22). Their amplitude is readily changed, generally reciprocally to the peristaltic excursions (figs. 27 and 30). The pendulum movements were the predominant type in the fresh, clipped preparations, but in the L segments they could be changed to the peristaltic type by moderate cooling (fig. 27). Cooling below 25°C. restores the pendulum type. Aeration, mechanical stimulation (bubbling) or asphyxia do not change the pendulum type to peristaltic (fig. 21), nor the peristaltic to pendulum (fig. 23); nor does increase or decrease of load effect this transformation (fig. 19).

2. Peristaltic type. This consists of slower and generally stronger rhythmic tone waves corresponding to the peristaltic movements in intact intestines and which may therefore be called peristaltic movements. They form major waves on which the pendulum contractions are superimposed as minor wavelets (fig. 27 long). In general, the amplitude of the peristaltic and pendulum waves vary inversely to each other (fig. 27 long); but they are parallel with load and aeration and sometimes for temperature. The peristaltic rhythm is less regular than the pendulum, and its rate is subject to wide variations, from less than one (fig. 10 long.) to 3 per minute (fig. 11 long.). Table 1 shows that the rate of the peristaltic waves averages the same in the longitudinal and in the circular limb and in unclipped, fresh and iced intestine; but that it is more rapid in the clipped than in the unclipped preparations. Apparently therefore the presence of the mucosa restrains the rate of peristalsis. On the other hand, the mucosa favors

TABLE 1

Rate of peristaltic waves (per 5 minutes)

These counts were made on a few typical tracings, selected rather at hazard.

FRESH CLIPPED INTESTINE		ICED INTESTINE	FRESH UNCLIPPED INTESTINE	
Circular limb	Longitudinal limb	Longitudinal limb	Circular limb	Longitudinal limb
8	5	6	6	6
12	8	7	7	6
6	10	4		6
8	10	7		7
12	11	6		
7		6		
Average 9	9	6	6½	6¼

the appearance, i.e., the amplitude of the peristalsis. This may be due to the presence of the submucosal ganglion plexus which may favor the amplitude of the peristalsis, perhaps by modifying the muscular tone; but the effect of the mucosa is only quantitative, for good peristaltic contractions may be evoked in clipped preparations, for instance by slight cooling (fig. 26).

The rate of peristalsis is increased by warming (fig. 28) and by load (fig. 20). Warming also increases the amplitude of the peristaltic waves (fig. 27), but with load the rate and amplitude of peristaltic waves are inverse to each other (fig. 20) and this applies also to changes that occur spontaneously or through drugs.

There is generally a striking parallelism between tone and the appearance or amplitude of the peristaltic waves; presumably because they are concurrent expressions of increased excitability.

3. Rhythmic twitches. These are rapid, maximal contractions, occurring with imperfect rhythm, at about the same intervals as peristaltic waves, i.e., one-half to two or three minutes. They are rather common in the circular limb of the iced intestines (fig. 12); in the longitudinal limb they occur imperfectly and only at the beginning of the contractions in iced preparations (fig. 10). This type of rhythm is probably due to a prolonged refractory state following a maximal twitch, in intestines that are relatively sluggish, and therefore predisposed toward refraction. The icing and partial asphyxia of the unclipped limb would be likely to develop these conditions. Figure 13 shows in the circular limb of the fresh unclipped preparation the abortive attempts at a pendulum rhythm between the twitches. As the excitability increases (temporarily) these abortive attempts become effective and the twitch type thus becomes a pendulum type. The right of figure 12 shows the relation of the twitch type in the circular to peristaltic waves in the longitudinal limb: apparently the whole contractile energy which goes into the slow peristaltic contractions of the longitudinal limb, is expended in the rapid twitch of the circular limb.

4. Spasmodic tone. This is generally a manifestation of the exaggerated peristaltic activity, of such rate or degree that the relaxation of the muscle becomes incomplete (figs. 10 and 24). The amplitude of the individual peristaltic waves and of the pendulum contractions is diminished. The spasm is usually sustained but occasionally occurs as long waves. Partial asphyxia, for instance, indirect bubbling, sometimes results in spasm, which may then be relieved by direct bubbling (fig. 24). A special instance of spasmodic tone is the heat spasm which occurs about 45°C. (fig. 29).

5. Tonic rigor. This occurs particularly in unclipped circular limbs and is attributed to asphyxia. The muscle shortens gradually but greatly, without rhythm (fig. 10); if rhythmic contractions have been present, they become weaker and disappear (fig. 14) and the intestine becomes inexcitable.

6. Suspension of contractions. Total cessation of contractions, with subsequent complete restoration of rhythmicity, was observed only under two conditions: in the "dormant period" of iced intestines, and on abrupt cooling. It may be recalled that intestines which have been kept in the refrigerator, when transferred to the warmed aerated bath execute a few vigorous twitches, but then remain entirely quiescent for a long period, one half to one hour for the longitudinal limb, one to three hours for the circular, and then suddenly or gradually resume vigorous and regular rhythmic movements (fig. 12). During this sojourn in the refrigerator the intestines have been subjected to prolonged asphyxia and cold. Our experiments do not decide directly between these two explanations; but from the completeness of the ultimate recovery we would be inclined to attribute the dormancy to the persistent effects of the preceding cold, which were found to disappear rather slowly even in acute experiments.

GRADATION OF THE STIMULATING AND DEPRESSING AGENCIES. Attempts to arrange the conditions in the order of relative stimulation or depression present some difficulty, because the various functions are not affected in the same degree, and sometimes not even in the same direction. In this manner, the conditions which we have studied gave combinations of effects, which we would arrange as follows:

Class A. Increase of peristaltic rate

	Effects	*Conditions*
Group I	Increase in all functions, i.e., of peristaltic rate and amplitude, of tone, and of pendulum amplitude	Direct bubbling Warming circular through optimal range (30 to 42°)
Group II	Increase of peristaltic rate and amplitude and of tone Decrease of pendulum movements	Warming longitudinal and circular below the optimal range (20 to 36°)
Group III	Increase of peristaltic rate Depression of peristaltic amplitude, of tone and of pendulum movements	Load Warming longitudinal in physiological range (30–42°)

Class B. Decrease of peristaltic rate, increase of peristaltic amplitude

Group IV	Increase of peristaltic amplitude and tone	Intact mucosa, fresh or iced
	Decrease of peristaltic rate and of pendulum amplitude	Cooling of longitudinal to 30°C.
Group V	Increase of peristaltic amplitude Decrease of peristaltic rate, of tone and of pendulum amplitude	Abrupt cooling of longitudinal

Class C. Decrease of peristaltic rate and amplitude

Group VI	Decrease of all functions except tone	Tonic spasm Heat spasm Tonic rigor of circular (intact mucosa)
Group VII	Decrease of all functions except pendulum amplitude	Abrupt heating Cooling below 25°
Group VIII	Decrease of all functions	Abrupt cooling of circular

CONCLUSIONS

1. Previous methods for studying simultaneously the longitudinal and circular muscle of the intestines tend to give misleading results because of mutual distortion.

2. This interference may be avoided by using an L-shaped segment.

3. The study of the circular muscle requires the removal of the mucous membrane to prevent asphyxiation, which results in tonic rigor.

4. The contractions of the longitudinal and circular muscles of rabbit's ileum present strikingly similar phenomena and they behave similarly but not always identically to changes of condition. The circular appears more responsive to the various changes.

5. In a given L-shaped segment, the contractions of the longitudinal and circular muscle may be of the same or of different types; but even if they are of the same type, there is no correlation between the two segments; one or the other may take the lead, from one minute to the other; and a double contraction in the one is not reflected by a double contraction of the other. It appears, therefore improbable that there is any neuromuscular coördination between the two limbs.

6. In the movements of the intact intestine a coördination would undoubtedly occur through mechanical stimulation: The contraction of either muscle would exert traction or pressure on the other and therefore act as an effective stimulation.

7. Stretching of the muscle by a moderate load favors the initiation and continuance of the rhythmic contractions. The beneficial load is confined to a rather narrow range. Stretching of the muscle by increased load diminishes the amplitude of the pendulum and peristaltic excursions and increases the peristaltic rate.

8. Agitation of the bath, by bubbling indifferent gases and otherwise, acts in the same direction as aeration, by bringing the oxygen in the solution into more effective contact with the muscle.

9. The effects of gradual cooling and warming differ materially for the longitudinal and circular muscles, and cannot be reduced to a simple schema; except that the rate of the pendulum movements varies in the direction of the temperature. Within the range of changes that might occur in the intact mammals, cooling slightly depresses the circular muscle but greatly increases the tone of the longitudinal muscle, and tends to start or to increase its peristalsis. Moderate warming above the normal somewhat stimulates the circular and depresses the longitudinal muscle. At a critical temperature, about 42 to 44°C., both muscles go into tonic spasm. The tone and peristalsis of the longitudinal muscle are at their maximum about 30°, and are depressed by further cooling. The effects of abrupt changes of temperature are even more complex.

10. The pendulum movements and the peristaltic waves react quite differently to variations of conditions. The pendulum rate remains constant, except with temperature; the amplitude is easily altered. The peristaltic waves vary in rate as well as in amplitude, but there is often a reciprocal relation between these, and also between the amplitude of the

peristaltic waves and of the pendulum movements. There is generally marked parallelism of tone and peristaltic activity, either rate or amplitude or both; but excessive spasmodic tone interferes with peristaltic relaxation.

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PROGRESSIVE CHANGES IN THE EXCITABILITY AND TONE OF EXCISED INTESTINES (MAGNUS METHOD) AND THEIR INFLUENCE ON THE RESPONSE TO PEPTONE

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In the course of an investigation of the effects of bacterial toxins on the intestinal movements, it became desirable to observe the direct actions of bacterial cultures on excised rabbit's intestine. To do this it was necessary to check the apparent effects of the culture against those of peptone which was a constituent of the bouillon, and which is known to affect the intestinal muscle. Since the response varies considerably with different pieces of intestine (Olivecrona, 1921) it was planned to apply the peptone and the toxin successively to the same piece of intestine; but it was found that after washing out the peptone, a second application of the same concentration of the same peptone in the same intestine did not produce the same effects as the first application. The same modification of response was found to occur toward the first application of the peptone if this was delayed for an equal interval of time; i.e., the exposure of the intestine in the saline bath sufficed to alter its response. This prompted a study of the changes that the movements and tone of the Magnus preparation undergo spontaneously on prolonged immersion, and it was found that these parallel the changes in the response to peptone. The observations emphasize the need of taking account of the progressive spontaneous changes in the Magnus preparations not only in quantitative, but even in qualitative studies; and especially the need of caution in attempting to standardize reactions by repeated application to the same piece of intestine.

METHOD. The rabbits were killed by a blow on the neck, pieces of duodenum and jejunum were cut out immediately and transferred to aerated Locke solution at 38°C.; or cooled to about 4.5°C. for iced intestine. The warmed Locke solution was aerated continuously until the segment was transferred to the bath of the recording apparatus, which contained 180 to 200 cc. of warmed Locke solution and which was also continuously aerated. The simple Magnus arrangement was used, the tubular segment of the

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intestine being attached between an L shaped support and a muscle lever. The latter was balanced to exert a tension of 0.5 gm. in air. The Locke solution had the composition (gram per liter): NaCl, 9.0; KCl, 0.42; $CaCl_2 \cdot 2H_2O$, 0.24; $NaHCO_3$, 0.3; glucose, 1.0. Under these conditions the movements were purely of the pendulum type.

The effects of peptone on fresh intestinal strips. The fresh intestines were allowed to record for half to one and one-half hours, until the contractions had become constant. A filtered 2 per cent solution of Witte's "Peptonum Siccum" in warmed Locke's fluid was then added to the 200 cc. bath; so that 1 cc. of the peptone solution gave a concentration of 1:10,000. With such intestines, 1:20,000 generally produces a slight rise of tone, which however is much more marked with 1:10,000 (fig. 1A). The amplitude of the excursions generally decreases reciprocally. The tone reaches its acme in six to eight minutes, and then usually returns gradually to the original level or lower and the pendulum movements tend to become progressively smaller as in figure 1. This depression, however, varies greatly in different experiments. Higher concentrations tend to produce a somewhat but not much greater rise (compare fig. 2, 1:2500, fig. 3, 1:1000). Intestines which have been iced for three hours respond very similarly.

Repeated applications of peptone. Successive additions of peptone, in close succession, repeat or rather increase the tone-rise of the first administration, as illustrated in figure 1A. However, when the peptone solution was replaced by unpoisoned Locke solution, and a half hour or longer elapsed to permit recovery to normal, and peptone was then reapplied, the response was altogether different, as is illustrated in figure 1B which was taken from the same piece of intestine. The concentration of 1:3500 produced only a very slight rise in tone, followed promptly by marked and progressive fall of tone and diminished pendulum movements. The altered response might have been due to the previous application of peptone, as suggested by Thienes, 1926; but it was also conceivable that the response of the intestines may gradually alter spontaneously after it has been removed from the body. This was tested in the following manner.

Change of peptone-response by prolonged sojourns in the warmed aerated Locke solution. This was investigated by cutting four or five adjacent segments from the upper jejunum, placing these in warmed aerated Locke solution, and removing a segment at intervals of zero to three hours, allowing it to trace for one-half to three-quarters hour, and then adding the peptone in concentrations of 1:5000 to 1000. This exposed pieces of intestine from the same general level of the given animal, but in varying order, to a single application of peptone, after they had been from one-half to three and a half hours in the bath. Four such series of experiments were made on fresh intestines, and one on iced. The results were practically uniform, as illustrated in figure 2: the tone-rise response increases for about three-

quarters ($\frac{1}{2}$ to $1\frac{1}{2}$) hours, and then diminishes rapidly and markedly, so that concentrations below 1:5000 produce no rise, slightly higher concentrations produce a smaller rise, of shorter duration, and often followed by a fall. In figure 3 the first two tracings represent different segments, one *A* after one and one-half, the other *B* after two hours of immersion; the latter segment was then washed and again exposed to the peptone, three and three-quarters hours after excision, *C;* then again washed and again exposed, five hours after excision, *D*. It is quite apparent that the four tracings represent successive steps of the same change, confirming that the changes are due merely to the time of immersion and that the previous application of peptone has essentially no effect on the response.

The progressive changes of the pendulum movements of the Magnus preparations in the aerated warm Locke bath. Figures 1, 2 and especially 3, showed progressive changes in the pendulum movements before the application of the peptone, which would presumably have an important bearing on the altered peptone response. To obtain a complete picture of these spontaneous changes, unpoisoned segments suspended within ten minutes after opening the abdomen were allowed to trace their full course on slow drums. Eleven experiments with the fresh intestines show essentially the same features, illustrated in figure 4*A*. They consist of four stages:

1. A moderate decrease of tone with increased amplitude of the pendulum movements. The tone reaches its minimum in about one-half to one hour; then,

2. Fairly abruptly the tone begins to rise considerably, with reciprocal

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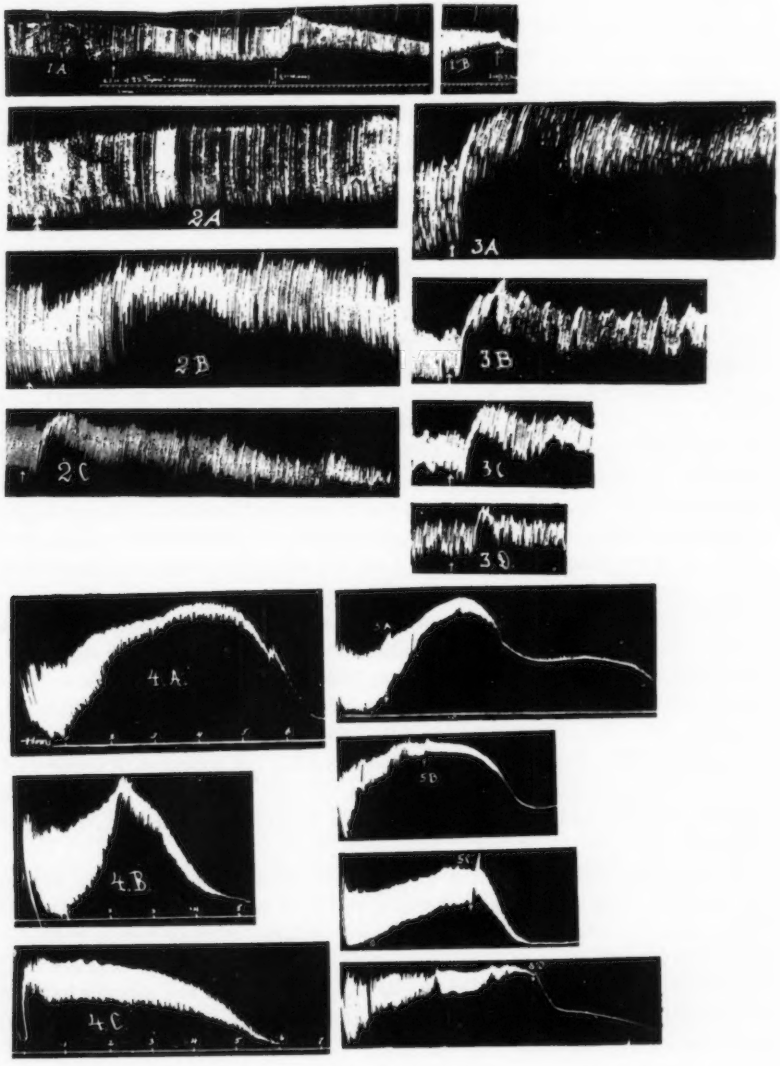
Fig. 1. *Successive applications of Witte's peptone to excised jejunum of rabbit (Magnus preparation):* Tracing *A*, at first arrow peptone 1:20,000 is added after intestine had been tracing about an hour in the warmed aerated Locke solution; at the second arrow, the concentration is raised to 1:10,000. The intestine is then allowed to run about an hour in the unpoisoned Locke solution, when peptone 1:3,300 is added in the tracing B. The time tracing is in minutes.

Fig. 2. *The effect of the sojourn in the Locke solution on the response to peptone.* The tracings represent adjacent loops from the same rabbit, which had been in the warmed aerated solution for various intervals of time before the peptone, 1:2500, was applied (at the arrows), namely: *A*, $\frac{1}{2}$ hour; *B*, $1\frac{1}{2}$ hours; C, 3 hours.

Fig. 3. *Identity of changes of peptone response by time, with and without washing:* Peptone 1:1000 was added as follows: *A*, $1\frac{1}{2}$ hours after immersion; *B*, another loop, 2 hours after immersion. This loop was then placed in unpoisoned Locke solution for one and three quarters hours, (total time, $3\frac{3}{4}$ hours after immersion), when the peptone was applied at *C*. It was again placed in unpoisoned solution for $1\frac{1}{2}$ hour (total time, 5 hours after immersion), when tracing *D* was taken.

Fig. 4. *Slow tracing of intestines in unpoisoned warmed aerated Locke solutions. A*, typical course of fresh segment; *B*, very short plateau; *C*, typical course of intestine that had been kept in iced Locke solution for 30 hours before transferring to the warmed solution.

Fig. 5. *Peptone 1:2500 applied at various phases of the slow tracings.*



Figs. 1–5

decrease of the amplitude of the pendulum movements. This reaches its maximum in one to two hours after immersion.

3. The tone then tends to remain almost level, or may rise or fall quite gradually. The amplitude becomes gradually smaller. This plateau is of quite varying length, that of figure 4*A* corresponding fairly well to the composite of the eleven experiments; but it may be somewhat longer or considerably shorter, as in figure 4*B*.

4. Diminishing of tone to about the original level, with a rapid parallel decrease of amplitude to zero, the contractions being arrested four to seven hours after immersion.

The tracing has the same general form whether the bubbles of air are directed against or away from the intestine, although changing the air current during the course of the tracing produces the usual acute alterations.

Iced intestines, that is, segments which have been in refrigerated Locke solution for thirty hours, take a somewhat different course during the first half of the tracing, as illustrated in figure 4*C*, which corresponds closely to the composite of the nine experiments. The iced intestines show the preliminary fall of tone, but the rise of tone in the second stage is much more abrupt than in the fresh preparation, due doubtless to the summation of the recovery from cooling. The final fall of tone occurs more gradually, so that the plateau is lengthened at both ends.

The curve of the Magnus preparation is somewhat different from that of the longitudinal and circular limbs of unclipped L shaped segments, fresh and iced (Šiaulis and Sollmann, 1927). According to these experiments the circular muscle of the Magnus preparations must be asphyxiated, so that it goes into asphyxial rigor, with progressive rise of tone and absence of rhythm. This must be partly responsible for the tone-rise of the second stage. The pendulum movements must be due exclusively to the longitudinal muscle, and this is probably also partly concerned in the tone changes.

As the tone and movements of the intestine are altered greatly during the course of the four stages through which it passes, its excitability and its response to drugs must evidently also undergo corresponding changes; and what is perhaps even more important, these spontaneous changes may easily be mistaken for reactions to poisons, especially if the poisons happen to be applied near the "critical points," that is, between the first and second and between the third and fourth stage. The safest periods for the experiments would be in the first half of the plateau, that is between one and one-half and two hours, in the fresh intestines, and between one and one-half and three hours in the iced preparations.

Relation of the peptone response to the spontaneous changes in the intestines. This was determined on the basis of the experience that had been gained, by taking slow tracings from another series of ten segments, to which

peptone, 1:2500, was added at different stages of the curve. The effects are illustrated by figure 5. It will be seen that the addition of peptone during the ascent of tone (*A*, ¾ hour after immersion), and in the middle of the plateau (*B*, 3 hours after immersion) cause about equal rise of tone; but when added toward the end of the plateau (*C*, 3 hours after immersion) the tone rise becomes short and precipitates the decline of tone and when added during the descent (*D*, 4 hours after immersion) the tone rise is slight and very short, and the decline abrupt. In other words, in the first half of the curve, with ascending tone, the tonic effect of the peptone predominates and with this concentration the action is purely tonic. In the second part of the curve, toward and during the descent of tone, the tonic effect becomes shorter and smaller and precipitates rapid diminution of the excursions and abrupt fall of the tone.

We are not prepared to discuss in detail the cause of the altered reactions. We may mention that Gunn and Underhill, 1914, found that intestinal segments kept in warm Locke solution became irresponsive to pilocarpine and atropine after three and one-half hours, while epinephrin and barium were still effective. This might suggest that the parasympathetic mechanism degenerates in the bath before the sympathetic and "muscular" excitation. However, the altered response to peptone, which presumably does not act on the parasympathetic mechanism, suggests that the changes in the susceptibility to these drugs may be due to the manner rather than to the site of their action.

CONCLUSIONS

Loops of intestine suspended in warmed aerated Locke solution undergo a complex but constant series of progressive changes in tone and in the amplitude of the pendulum movements. These must be borne in mind in interpreting the apparent response of fresh loops to poisons, especially when these are applied near the two "critical points" at which the tone reverses; or when several applications of poisons are separated by considerable intervals of time. The alterations of response are illustrated by "Peptone." When applied in the first two hours after immersion, whilst the tone is rising, this produces a pure rise of tone, with diminished excursions. About three hours after immersion the intestine becomes much less responsive to the tone-rise; this requires larger doses, is of short duration, and precipitates or accentuates the descent of tone.

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THE PREVENTION OF TETANY BY THE ORAL ADMINISTRATION OF AMMONIUM CHLORIDE

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Since the pioneer work of MacCallum and Voegtlin (1909), many salts have been used for relief of parathyroid tetany. These investigators, however, were the first to show that the blood of parathyroidectomized dogs contained less calcium than the controls,and that the administration of calcium salts temporarily relieved the tetany produced by parathyroidectomy. Later, Joseph and Meltzer (1910–1911) showed that intravenous injections of sodium chloride was efficacious. Strontium lactate (Swingle and Wenner, 1926)(Dragstedt, 1925) and magnesium lactate (Wenner, 1927), given orally, have been used successfully in the treatment of tetany following the extirpation of the parathyroids in dogs.

The lactate and chloride of calcium were used, but of the two, the chloride appeared to be the more efficacious. Haldane, Hill and Luck (1923) showed that the ingestion of large amounts of calcium chloride causes a change in the reaction of the blood toward the acid side. The calcium passes through the alimentary tract in the form of calcium carbonate and the chlorine, almost completely absorbed, replaces the bicarbonate in the body causing acidosis.

Wilson, Stearns and Thurlow (1915) reported that injection of 0.1 to 0.5 gram of hydrochloric acid brought relief to dogs following the onset of parathyroid tetany. Acid producing salts, such as $CaCl_2$ and NH_4Cl, as shown by György (1922), Gamble and Ross (1923) and Anderson and Graham (1924), are effective agents in the treatment of infantile tetany.

The present work was undertaken with the idea of determining the effect of NH_4Cl on the prevention of experimental tetany and the action of this acid-producing salt on the serum calcium in parathyroidectomized dogs. The series of experiments reported here was completed in the spring of 1926, but publication was delayed a year owing to the fact that this work, together with another paper (Wenner, 1927), constitutes a part of the requirements for the Ph.D. degree, the other requirements for which could not be met until the spring of 1927. During the interim, a paper appeared by Boyd, Austin and Ducey (1926), which dealt with somewhat similar investigations.

Boyd and his associates used seven dogs, three of which were fed on liver and bread, and four on bread and milk. Ammonium chloride in 2 per cent solution was administered by mouth. All three of the dogs on a liver and bread diet died at the end of 26, 20 and 10 days. Three of the four dogs on the bread and milk diet died at the end of 26, 27 and 39 days. One dog survived 197 days. Boyd, Austin and Ducey state that the survival period of dogs receiving ammonium chloride is considerably prolonged but recovery as a rule does not occur.

The writer takes pleasure in acknowledging his indebtedness to Prof. W. W. Swingle for suggesting the problem, and for much helpful advice and criticism.

Six dogs used in this investigation were thyroparathyroidectomized according to the technique described in an earlier paper (Wenner, 1927). The parathyroid glands were identified before and after the thyroids and surrounding fascia were excised. All dogs, with the exception of dog 1, developed violent tetany some time following operation. Great care was taken to see that all of the parathyroid tissue was removed.

Ammonium chloride in five per cent solution was given in 50 to 100 cc. doses twice daily by stomach tube. The solution is retained if given four to five hours before and five to eight hours after feeding.

The data of two of the six experimental dogs are summarized in table 1.

The serum calcium was determined by the Clark and Collip (1925) modification of the Kramer and Tisdall (1921) method. The serum calcium curves of dogs 3 and 5 show the effect of ammonium chloride on the level of the calcium. Dog 2, plate 1, did not develop tetany until after eating a large portion of meat on the thirty-ninth day. Animal 3, figure 1, received no medication until after the first appearance of tetany. Tetany developed two days after operation and following the administration of 5 grams of ammonium chloride, all tetany symptoms disappeared. The next day the serum calcium was found to be 10.6 mgm. or an increase of 3.3 mgm. in 24 hours. Eight days later the dog again developed violent tetany, serum calcium was 6.1 mgm. One and one-half hours after receiving 100 cc. of a 5 per cent solution of NH_4Cl all tetany symptoms disappeared and the calcium rose to 8.5 mgm. per 100 cc. One hundred to 200 cc. of a 5 per cent solution of NH_4Cl given by stomach tube to a dog in violent tetany brought about a recovery within $1\frac{1}{2}$ hours. Figure 5 shows dog 4 in a prostrate condition, all skeletal muscles were twitching, extensor muscles rigid. Figure 6 shows the same animal $1\frac{1}{4}$ hours after receiving 100 cc. of a 5 per cent solution of NH_4Cl.

Animals 1, 2, 5 and 6 recovered permanently after periods of NH_4Cl therapy lasting from two weeks to forty-one days. These dogs were placed on a full meat diet at the end of that time and never again developed tetany. Animal 3, however, was placed on a meat diet, NH_4Cl discon-

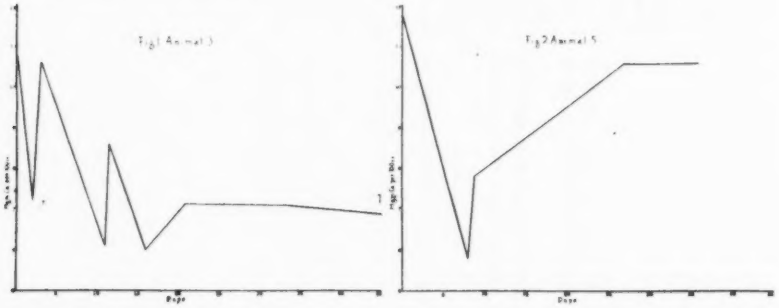
TABLE 1

ANIMAL	DATE	TIME	SERUM CALCIUM	AMMONIUM CHLORIDE GIVEN	REMARKS
	1926		*mgm. per 100 cc.*		
Dog 2					
Adult male	April 10	10:30 a.m.	10.9		Thyroparathyroidectomy
10 kilos		6:00 p.m.		100 cc. 5 per cent solution	
	April 11–14			100 cc. 5 per cent solution	Normal
	April 15		10.9	100 cc. 5 per cent solution	No tetany
	April 16–17			50 cc. 5 per cent solution	Normal
	April 18				No NH_4Cl given. Enema
	April 19			100 cc. 5 per cent solution	Normal
	April 20–23				Good condition. No NH_4Cl
	April 24		9.3	100 cc. 5 per cent solution	Slight twitching of muscles of flank
	April 25–May 17			50 cc. 5 per cent solution	No tetany
	May 18	9:00 p.m.	9.5		Fed large portion of meat
	May 19	2:00 p.m.	6.0	100 cc. 5 per cent solution	Violent tetany, hyperpnea, extensor rigidity
		3:15 p.m.			Recovered. Tetany entirely disappeared.
		6:00 p.m.		100 cc. 5 per cent solution	Normal
	May 20			100 cc. 5 per cent solution	Normal
	May 21				Placed on meat diet. No NH_4Cl given
	May 29		10.3		Normal
	June 12				Normal. Used for other experiments

Dog 6					
Young airedale	May 28		10.2		Thyroparathyroidectomy
9 kilos					
	May 29				No tetany
	May 30				Slight muscle tremors respiration increased
	May 31	5:15 p.m.	6.6		Violent tetany
		5:30 p.m.		100 cc. 5 per cent solution	
		7:00 p.m.		100 cc. 5 per cent solution	All tetany symptoms gone
		9:30 p.m.	10.1		Dog apparently normal
	June 1– 6			200 cc. 5 per cent solution	Normal
	June 7–10			100 cc. 5 per cent solution	Good condition
	June 11				Taken off NH_4Cl therapy. Fed meat
	July 3				Used for other purposes

tinued, June 14—thirty-one days after operation, and fourteen days later died of tetany and pneumonia.

From the results obtained with ammonium chloride it appeared that the anion of strontium and magnesium lactate used in previous experiments, may probably have been equally effective as the cation in preventing tetany. Consequently calcium-free 1 to 5 per cent lactic acid milk was tried. Three parathyroidectomized dogs received daily one quart of lactic acid milk by stomach tube immediately following operation. All three animals died within ten days to two weeks. However, these negative findings do not indicate that lactic acid is entirely ineffective. It will be recalled that Dragstedt and Peacock (1923) placed their experimental animals on the tetany preventing lactose diet four to six days before operation. None of the three dogs treated with lactic acid received any pre-operative treatment.



Figs. 1–2. Serum calcium curves of dogs 3 and 5 showing the effect of ammonium chloride on the level of the calcium.

The use of hydrochloric acid (Wilson, Stearns and Thurlow, 1915) and acid producing substances (Freudenberg and György, 1922; Gamble and Ross, 1923) supported the view that a condition of alkalosis is present during tetany. The recent investigations of Drucker and Faber (1926) on infantile tetany show that infantile tetany is not due to a change in the reaction of the blood in an alkaline direction. Wenner and Muntwyler (1927) have clearly demonstrated that a condition of alkalosis does not exist at any period following parathyroidectomy in dogs. The carbon dioxide content of the plasma, and the hydrogen ion concentration remain within normal range. After recovery from convulsions the pH and alkali reserve of the blood may fall considerably. At this low pH, 7.09, and low CO_2 content, 26.8 volumes per cent, all tetany symptoms disappeared.

In studying the effect of withdrawal of considerable quantities of blood from dogs suffering from tetania parathyropriva, Swingle and Wenner (1926) found that such procedure causes a temporary abatement of all

tetany symptoms coincident with a marked rise in blood calcium within 1½ to 7 hours. Tetany, along with the fall in the calcium, recurs on the

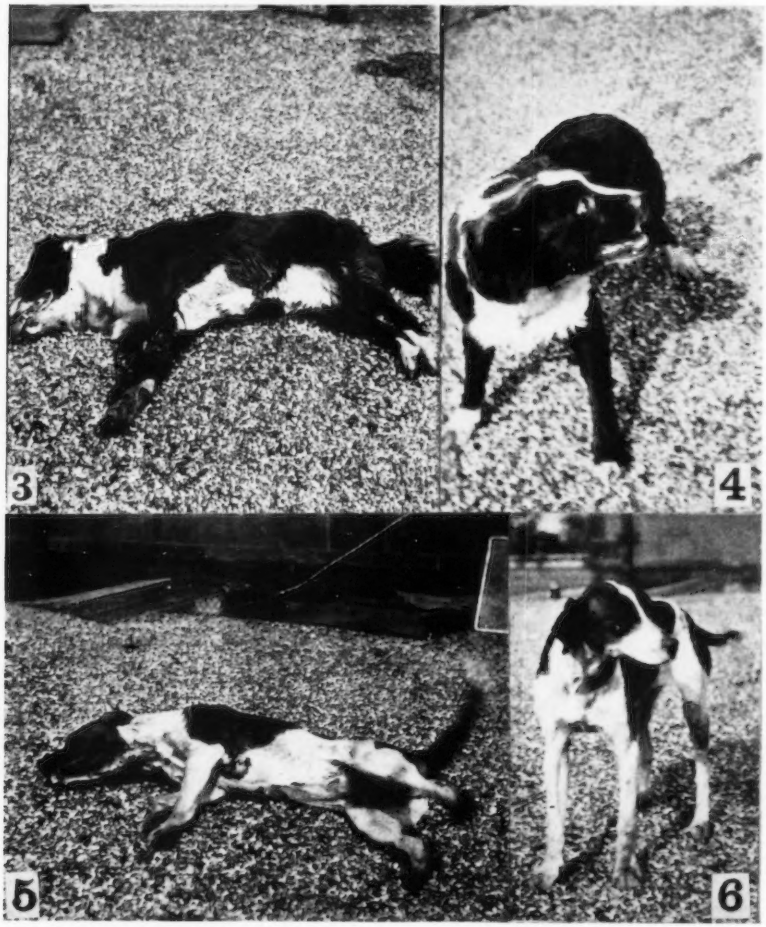


Fig. 3. Animal 2 in violent tetany.

Fig. 4. Dog 2, 1¼ hours after receiving 100 cc. of a 5 per cent solution of ammonium chloride.

Fig. 5. Dog 4 in violent tetany.

Fig. 6. Dog 4, 1¼ hours later after having received 100 cc. of a 5 per cent solution of ammonium chloride.

following day. Later, Bennett (1926) observed that the removal of one-fourth to one-half of the blood of normal dogs results in a fall of 0.02 to

0.12 pH within four or five hours. On the following day the pH was 0.01 to 0.11 above normal values. It is probable that the rise in calcium produced by bleeding was the direct result of the change in the reaction of the blood toward the acid side.

Binger (1917) was able to show that a reduction in the calcium content of the blood occurs after injections of both acid and alkali phosphates. Tetany accompanied the fall in calcium only when the pH of the phosphate injected was 5.6 or greater. Orthophosphoric acid and acid phosphates produced a decrease in the calcium but tetany did not occur.

The importance of acidity in relation to calcium absorption has been indicated by the interesting work of Steenbock, Hart, Sell and Jones (1923) who found that calcium salts soluble in dilute acids, are absorbed in the intestine because the acid chyme is only gradually neutralized at the pyloric region. A decrease in gastric acidity, affording a poor calcium solution medium and therefore causing a decrease in calcium absorption, has been offered as a suggestion by Babbott, Johnston and Haskins (1923) as a possible explanation for the mechanism in the production of many cases of tetany in infants.

These observations tend to indicate that the administration of NH_4Cl, by increasing HCl metabolism and thereby decreasing the pH of the blood, determines the solubility of the calcium. Greenwald and Gross (1925) owing to their failure to observe an increase in calcium excretion in parathyroidectomized dogs, suggested that calcium is precipitated in the tissues as calcium phosphate, which in the absence of the parathyroid hormone becomes less soluble. With the increase in the acid radicle it is possible that this salt is brought into solution.

The permanent recovery after thirty to forty days' treatment with ammonium chloride was certainly not the result of any failure to remove all parathyroids at the time of operation. Out of nearly two hundred dogs operated on during the past few years only three have failed to develop tetany following complete removal of the parathyroid glands. It is true that accessory parathyroids occur in a large percentage of cats; Nicholas and Swingle (1924–25) found accessory parathyroids in 35 per cent of these animals, but in dogs, however, accessory glands occur less frequently. Marine (1914) states that only 5 to 6 per cent of dogs were found to possess them. Of the forty dogs used as controls, and for experiments on potassium, sodium and cadmium lactate, lactic acid and lactic acid milk, all died of tetany within two weeks. The same careful technique was used for all operations.

The mechanism of the readjustment that takes place within the body after forty days' treatment with strontium lactate (Swingle and Wenner, 1926), magnesium lactate (Wenner, 1927) and ammonium chloride has been discussed in an earlier paper (Wenner, 1927) where it was suggested

that the mechanism of adjustment may involve 1, the hypertrophy of accessory parathyroid tissue, or 2, the taking over of the function of the parathyroid glands by other organs. However, it should be stated that no evidence exists at present in favor of either suggestion in so far as dogs are concerned.

SUMMARY

1. Ammonium chloride administered orally is efficacious in the prevention of tetany in parathyroidectomized dogs.

2. Dogs in violent tetany recover within one and one-half hours after receiving 100 to 200 cc. of a 5 per cent solution of ammonium chloride.

3. Thyroparathyroidectomized dogs receiving daily administrations of ammonium chloride for thirty to forty days become readjusted to the loss of the parathyroids and may be placed on a meat diet without ill effects.

4. Ammonium chloride probably exerts its action by rendering the blood more acid thereby producing a rise in the serum calcium and a disappearance of the symptoms of tetany.

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SEMI-OVARIECTOMY COMPENSATORY HYPERTROPHY OF THE REMAINING OVARY AND MIGRATION OF THE OVA IN THE ALBINO RAT[1]

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The purpose of this experiment was primarily to determine whether the ova could migrate to the opposite horn of the uterus in the albino rat, and secondarily to determine if there was a compensatory hypertrophy of the remaining ovary after semi-ovariectomy and also to ascertain the amount of intra-uterine mortality.

A large number of investigations have been carried on with a number of other animals and some important results obtained by different workers. The results and conclusions of a few follow.

King (1911) found in two semi-spayed females that "the remaining ovary appeared normal in every female, and there was no marked increase in its size to compensate for the loss of the other ovary." The ages of the rats were not given but the author states that "they were out of condition and would not breed again." From this we would infer that they were old rats which had passed the menopause. These results do not agree with those of other investigators.

Hatai (1913) says "In the semi-spayed series the compensatory growth of the remaining ovary is almost perfect and the single ovary has therefore nearly twice its normal weight." In a later paper (Hatai, 1915b) giving the results of a study of the growth of organs in the albino rat as affected by gonadectomy he confirms his former results. In this study he not only semi-spayed the animals, but also isolated the remaining ovary by separating its connection with the uterus. He found an average increase of 51.3 per cent in the surviving ovary. The somewhat less increase in size, as compared to his former results, he attributes to a possible disturbance of the blood supply to the organ.

Stotsenburg (1913a) also found a compensatory hypertrophy in semi-spayed albino rats. He says, "It is interesting to note that the remaining

[1] This research has been conducted with the aid of the Department of Physiology and the Research Fund of Stanford University, and The Committee for Research on Sex Problems of the National Research Council.

ovary thus appears to be able to exercise as full control as both ovaries together, but in doing this it hypertrophies to twice its normal size."

Arai (1920) finds the surviving ovary in semi-spayed albino rats larger than normal at all ages. Before puberty it is 40 per cent and after puberty 100 per cent or more than normal. The excess at the early age was due to a greater number of large follicles while in the older animals there was also an increase of corpora lutea. He found no evidence that the increased weight was due to stroma tissue. Semi-spaying did not interfere with normal development or in reproduction except that the number of ova discharged and young born were slightly less than normal.

Asdell (1924) semi-spayed rabbits and also removed various amounts of the remaining ovary so that in some cases as much as five-sixths of the total ovarian tissue was excised. He found that the size of the litter was but little smaller than normal, that the number of ova discharged was practically the same, that the sex of the offspring was not affected, and that the hypertrophy of the remaining ovarian tissue was proportionally greater as more ovarian tissue was removed. He found no case of migration of ova to the opposite horn and that fetal atrophy was greater in operated animals. This latter he attributes to overcrowding in the single horn of the uterus.

The hypertrophy of the remaining ovary following semi-ovariectomy in the rabbit to the extent of 55 to 80 per cent as compared with normal controls was found by Lipschütz, Wagner and Tamm (1922). Doncaster and Marshall (1910) found similar results in the rat. Other results confirming hypertrophy of the remaining ovary following unilateral ovariectomy might be given. King (1911) seems to stand alone in not finding a compensatory hypertrophy of the intact ovary following semi-spaying. Our results show an increased size of the unoperated ovary following the removal of the other and are thus in agreement with the majority of investigators.

It is generally thought that all the ova liberated from a given ovary find their way down the adjacent oviduct and, if fertilized, become implanted in the corresponding horn of the uterus when it is bicornuated. The number of ova liberated from a given ovary during a single oestrus is indicated by the number of corpora lutea found on the ovary. In animals with bicornuate uteri there often appears a discrepancy between the number of fetuses in one horn of the uterus as compared to the number of corpora lutea present in the corresponding ovary. In such animals there is usually found an almost equal distribution of the young in the two uterine horns. There are usually more corpora lutea than young which indicates a certain amount of intrauterine mortality. The presence of a greater number of young in a given horn than there are corpora lutea in the corresponding ovary shows that ova from the opposite ovary have found their

way across. The two possible means of accomplishing this are 1, ova may be discharged into the body cavity and enter the oviduct of the opposite side; and 2, they may migrate down through the adjacent oviduct and uterine horn to the bifurcation then up the opposite horn where they become implanted.

That the first manner is possible in the rabbit has been shown by Leopold (1880). He removed the ovary of one side and the uterine horn and tube of the opposite side. Later mating resulted in pregnancy. The only possible route for the ova from the isolated ovary to the fallopian tube of the opposite side was through the body cavity.

Experiments on the rabbit by Parkes (1924) have failed to substantiate the results of Leopold. Parkes performed ovariectomy on one side and salpingectomy on the other side of six rabbits. These animals were successfully mated a number of times for three or four months. In no case did pregnancy result and autopsies showed no indications of pregnancy. He concluded that external migration of the ova is very rare. Owing to the distance to travel and the lack of the power of locomotion on the part of the ova it is very doubtful if this sort of migration occurs often in mammals.

The second possible mode of migration mentioned above has been tested and found to occur in some mammals. It is especially likely to be found in animals which have bicornuate uteri.

Corner (1921) found in sows within three days following oestrus that the number of discharged ova of one side was the same as the number of corpora lutea of the same side. Later examination of the gravid uterus showed that this equality no longer existed and that there had been an adjustment toward equality of young in each horn. He concludes that this could have been accomplished only by a migration from one horn of the uterus to the other.

Warwick (1926) has collected extensive data in swine. Examination of 469 gravid uteri and ovaries gave 1936 fetuses and 2289 corpora lutea on the right side and 1932 fetuses and 2830 corpora lutea on the left side. This shows that the left ovary was more active and liberated approximately 10 per cent more ova than the right ovary. It also shows that migration from one side to the other occurred until there was practically an equal distribution of fetuses in the two horns. Warwick also bred semi-spayed sows and killed them at various ages of gestation. At autopsy all showed complete occlusion of the cut end of the tube on the operated side and removal of the corresponding ovary. In three sows examined the number of fetuses were 8, 9 and 5 respectively and their distribution was 4, 5 and 2 on the unoperated side and 4, 4 and 3 respectively on the operated side. We thus have absolute proof of intra-uterine migration of the ova in swine.

As shown above Asdell (1924) was unable to demonstrate a migration

of the ova from the unoperated to the operated side in semi-spayed rabbits. The differences in results in swine and rabbits is due to the morphology of the uterus in each animal. The swine has a bicornuate uterus so that the ova in migrating from one horn to the other never leave the uterine cavity. In the rabbit, however, there are in reality two uteri each of which has a separate opening into the vagina. The passage of ova from one horn to the other would therefore involve a portion of the vagina where they would most probably be discharged or lost.

In our experiment two groups of rats were used whose ages were but thirteen days apart. Each group consisted of four sisters. They were semi-ovariectomized on the same day at the ages of twenty-one and thirty-four days respectively. They had all been previously weaned. In each case the left ovary was excised. The operation caused very little, if any, disturbance, in their normal growth and apparently little inconvenience as they were playing and frolicking about as normal rats of this age the day following the operation. The following procedure was followed in each operation: The animal was anesthetized and fastened to the operating board dorsal side up with nose in ether cone for continuing anesthesia. The long hair was cut close with scissors over a region some 5 mm. wide and 10 mm. long over the lumbar triangle of Petit which is bounded by the iliac crest, latissimus dorsi and obliquus abdominis externus. The exposed skin was painted with alcohol and all instruments used were kept in alcohol. A small incision, 2 to 3 mm. long, was made through the skin parallel with the margin of the latissimus dorsi muscle and two pairs of small serrafine forceps served as skin retractors. A 1 mm. incision was now made through the thin body wall, a small glass hook inserted and the ovary and part of the Fallopian tube carefully pulled out. Before severing the ovary from its attachments the tube was pinched and bruised with forceps to prevent hemorrhage from the ovarian artery and to aid in healing and occluding the lumen of the tube. The ovary was now cut loose with one snip of the scissors, the tube returned to the body cavity, the two edges of the skin brought together without sutures and painted with a layer of collodion. The animals were then placed in a small box and covered with a cloth to keep them warm. In about a half-hour they were returned to their cages. The wound healed rapidly and without signs of infection.

We found, as other investigators have already shown, that semi-spaying did not in any way interfere with normal growth and development. We did find that there was a slight effect on sexual maturity as measured by the age at which the vaginal membrane opened, when compared with that of a group of double hysterectomized rats. The average age at opening of the vagina in twenty-eight hysterectomized rats was 43.75 days and in the eight semi-spayed animals it was 54.6 days. Both of these ages are considerably younger than the average of 72 days quoted by Donaldson

(1924) as given by Long and Evans (1922). The earlier development of our animals may possibly have been due to the food which they all received. They were fed continuously from the time they were weaned on the following synthetic diet: whole wheat, 3375; whole milk powder, 500; commercial casein, 750; sodium chloride, 50; calcium carbonate, 75; ground alfalfa, 150; butter fat, 250. These ingredients were thoroughly mixed and ground. Previous experiments had shown that rats fed on this diet had made rapid growth, had normal reproduction, were healthy, active, and lived to an old age. It apparently contains all the essential food factors.

The animals were all mated as nearly as possible at the same time and at the average age of 140 days. Successful insemination was determined by the presence of sperm in the vaginal smears. The exact hour of coitus was recorded which enabled us to determine closely the progress of gestation. The animals were weighed daily and the usual increase in weight characteristic of pregnant animals noted. The rats were killed one on the seventeenth, four on the eighteenth, one on the nineteenth, and two on the twenty-first day after mating and probably the same day of pregnancy and careful autopsies made. The results are condensed in table 1. The ages of the animals at death varied between 147 days and 168 days, the average being 161 days.

The results of the autopsies showed that all the animals were pregnant, that the left ovary had been completely excised and that complete occlusion of the Fallopian tube or uterus of the operated side had occurred.

All the fetuses were found on the unoperated side and there was no evidence of any implantation having occurred in the uterus on the operated side. This lack of migration of ova from the unoperated side to the operated side in the rat is in complete agreement with the results on the rabbit quoted above. The reason for this is the same in each animal. The two horns of the uterus join to form a common trunk before reaching the vagina. The cavities of the two horns, however, fail to unite to form a single tube like that found within the uterus of the pig, but each has its separate cervix. Like the rabbit, therefore the ova in migrating from one horn to the other would have to enter the vaginal cavity and then into the other horn. The chances of this occurring would, in my opinion, be very doubtful. The number of young varied from 2 to 9 and averaged 5.25 per rat. With one exception they were almost uniformly spaced along the uterine horn, the most distal being usually attached close to the cervix. The one exception was found in B4 which had 9 young crowded into the one horn, thus extending it to two or three times its normal length. Two of these young had a common oval placenta and as nearly as could be determined were contained in a common amniotic sac. They were attached to the common placenta by separate umbilical cords. These two

TABLE 1

Summary of results and averages. H. 1st and R. 1st, head or rump toward cervix

RAT	MATING		DEATH		GAIN IN WEIGHT	LENGTH LEFT UTERUS	FETUSES						OVARY			RESORPTION AREAS
	Weight	Age	Weight	Age			Number	Age	Total weight	Average weight	H. 1st	R. 1st	Weight	Corpora lutea	Number lost	
A1	189	149	237	167	48	25	6	18	4.60	0.76	3	3	0.12	9	3	1
A2	190	139	233	157	43	25	7	18	4.95	0.707				10	3	0
A3	176	142	219	160	43	20	2	19	2.60	1.30	1	1	0.10	7	5	0
A4	192	149	234	167	42	27	5	18	3.70	0.74	3	2	0.14	11	6	0
B1	173	144	213	161	40	35	5	17	2.10	0.42	3	2	0.10	8	3	0
B2	170	143	221	161	51	35	5	18	3.65	0.73	2	3	0.09	9	4	0
B3	174	126	218	147	44	23	3	21	9.30	3.10	1	2	0.15	7	4	0
B4	168	129	221	168	53	10	9	21	23.60	2.60	4	3	0.18	10	1	0
Totals	1432	1121	1796	1288	364	200	42	150	54.50				0.88	71	29	1
Average	179	140	224	161	45.5	25	5.25	19	6.81	1.3	17	16	0.126	8.9	3.625	0.125

were smaller than the remaining seven and averaged 1.85 grams while the others averaged 2.7 grams.

Of the total number of fetuses those whose head was toward the cervix exceeded those in the reverse position by only one. The effect of the size of the litter with consequent overcrowding on the weight of the young is shown by B3 and B4, table 1. This table also shows that the average number of young per litter was reduced from the normal of 6.9 to 5.25 which corresponds with the results of other investigators with semi-spayed animals. The average weight of the fetuses in our experiment is a little less than that given by Stotsenburg (1915). This may be partly explained by the fact that all our matings were made between 7 and 10:30 p. m. and that all the animals were killed in the morning. The ages of the fetuses would therefore lack from 10 to 16 hours of being the age in days we have recorded.

In order to determine to what extent mortality of the ova or young had occurred a careful count was made of the corpora lutea. A total of 71 corpora were found and since there were but 42 fetuses, there was a mortality of 29, or a little over 40.8 per cent. This is greater than about one-third given for normal rats by Long and Evans (1922). We had no means of determining how many of these lost ova had been fertilized, implanted and later resorbed since we found but one case of resorption. This was located close to the cervix and had all disappeared except a small blood clot, evidently the remains of the placenta, located in a shallow protuberance of the uterine wall. If implantation had occurred in large numbers, mortality and resorption had taken place during the early stages of gestation and left no evidence. The average number of ova liberated at an oestrus by the single ovary as indicated by the number of corpora lutea was 8.875. This is less than the average of 9.6 given by Long and Evans (1922) and corresponds in general with the results of other investigators.

The weight of the remaining ovary was found to vary in the different animals. In general those killed earliest in gestation had the lightest and those killed nearest the time of delivery had the heaviest ovaries. This agrees with the findings of Stotsenburg (1923). We also found that ovaries taken from animals in the same stage of gestation were heavier when a larger number of corpora lutea and greater number of fetuses were present than with a fewer number. The weight of the ovary therefore not only increases in weight with advancing pregnancy, but also with increasing numbers of corpora lutea. We found the average weight of the single ovary at the seventeenth day of gestation to be 0.10 gram, the eighteenth day 0.13 gram and the twentieth day 0.165 gram. Stotsenburg (1923c) found the combined weight of the two ovaries of normal females at the average age of 200 days for the corresponding days of gestation to

be 0.098, 0.13 and 0.122 gram respectively. We thus see that the single remaining ovary in our rats hypertrophied to more than twice the normal size.

SUMMARY

The following summary of the results of semi-ovariectomy in the albino rat may be given:

1. Semi-ovariectomy did not affect normal growth or activity, but slightly delayed the opening of the vagina.

2. A compensatory hypertrophy of the remaining ovary to twice or more the normal size followed semi-spaying of young females.

3. The weight of the ovary increased as gestation advanced. Its weight also varies directly with the number of corpora lutea present.

4. There was no evidence of migration from the unoperated to the operated side of the uterus.

5. The average number of corpora lutea was 8.875 as compared to the normal of 9.6.

6. The number of fetuses varied from 2 to 9, the average being 5.25. This was less than the normal average of 6.9. The average weight was but slightly less than normal.

8. But one case of resorption was found in the later days of pregnancy. This indicates that most of the mortality occurred at a sufficiently early time to allow for a complete eradication of the signs of resorption.

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OBSERVATIONS ON THE RÔLE OF TISSUES IN MAINTAINING THE ACID-BASE EQUILIBRIUM OF THE BLOOD

I. The Effect of Isolated Muscle Tissue

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The exact importance of the body tissues in maintaining the acid-base equilibrium of the blood has not been clearly established. There can be no doubt that tissues contain buffer systems, which cope with the acids accumulating during tissue activity pending their removal. A striated muscle, for example, goes on contracting until its lactic acid content reaches 0.25 to 0.30 gram per cent (Hill and Meyerhof, 1923), and yet the pH change is very small in the mammalian muscle (Katz, Kerridge and Long, 1925)—about pH 0.70 in cold-blooded muscle, according to Meyerhof and Lohmann (1926).

The mere fact that acids are buffered in the tissues does not necessarily indicate that the tissues aid in maintaining the acid-base equilibrium of the blood. The ready diffusion of CO_2 through tissue membranes is definitely established (Shaw, 1926) and that of the lactate radical indicated by the work of Hill, Long and Lupton (1925) and Barr, Himwich and Green (1923). The evidence regarding the diffusion of other acid or basic radicals, which might alter the acid-base equilibrium of the blood, is indirect and far from conclusive.

The changes in chloride and base content of plasma found by Peters, Bulger, Eisenman and Lee (1925), which they attributed to a shift of these ions between blood and tissue, may be equally well assigned to a redistribution of ions between plasma and red blood corpuscles. Experiments on intact animals, such as those of Henderson (1925) and Prentice, Lund and Harbo (1920) fail to control or evaluate the effect of the excretory power of the kidneys and lungs, the change in blood concentration, and the shift of CO_2 to the tissues.

These objections do not apply to the results of Atzler and Lehmann (1922), who found that the pH of acidified saline perfused through surviving mammalian tissue approached that of the perfused tissue. Two other serious objections, however, would invalidate the application of their re-

sults to the living animal: 1, the fact that they used a poor oxygen carrying perfusate; 2, that the pH of the perfusion fluids was far beyond the physiological limits to which living tissues might be exposed. Even were we to overlook these objections and accept their results as applicable to the question at hand, we should still be unable to judge whether or not tissues could increase the already large buffering capacity of whole blood, for they found that the changes in pH were least when the buffering capacity of the perfusate was largest.

In the present investigation we attempted to determine the extent, if any, of the acid-base interchange between the tissues and blood following the addition of acid to the whole blood. An isolated perfused organ, the gastrocnemius, was used, inasmuch as variations in alveolar CO_2 or in the elimination of acid by the kidneys, which tend to change the acid-base balance of the blood, could be eliminated. The changes in pH and CO_2 combining power, as well as the chloride and lactic acid content of the *whole* blood perfused through the gastrocnemius, were analyzed at various periods following the injection of hydrochloric acid. Any change that takes place under such circumstances must have occurred either as a result of an alteration in the perfusion blood or in the perfused organ.

Hydrochloric acid was chosen in preference to organic acids such as lactic, because: 1, the removal of anion by oxidation or synthesis did not have to be considered; 2, Cl' is an ion common to the body and shows facility in penetrating through the red blood cell membrane (Van Slyke, 1921); 3, the concentration of chlorides can be easily and accurately determined in small amounts of whole blood; 4, HCl has the advantage over organic acids that, due to its high degree of dissociation, much less of it is required to produce a given change in pH. *In this way the adaptations resulting from changes in pH per se can be dissociated from those arising in consequence of the presence of large amounts of undissociated acids.*

METHODS. *1. A new type of perfusion apparatus.* The perfusion system was built with the idea that a pulsating, well oxygenated stream of defibrinated or heparinized whole blood could be delivered to the perfused organ at a controllable mean pressure and CO_2 tension, at body temperature, and in an amount approximately the same as before removal from the body. The whole apparatus, except the pump and some of the motors, was enclosed in a constant temperature moist air chamber. The details are shown diagrammatically in figure 1.

The moist air chamber was warmed by four 50 watt carbon bulbs, *6*, controlled by an electric thermoregulator of the toluol-mercury type, *7*. The air of the box was kept in circulation by a small fan, *8*. Accessibility to the interior was made easy by making the top demountable in two parts and by placing a double glass door, *5*, in the front of the box. In the later experiments a smaller box with a glass cover was built onto

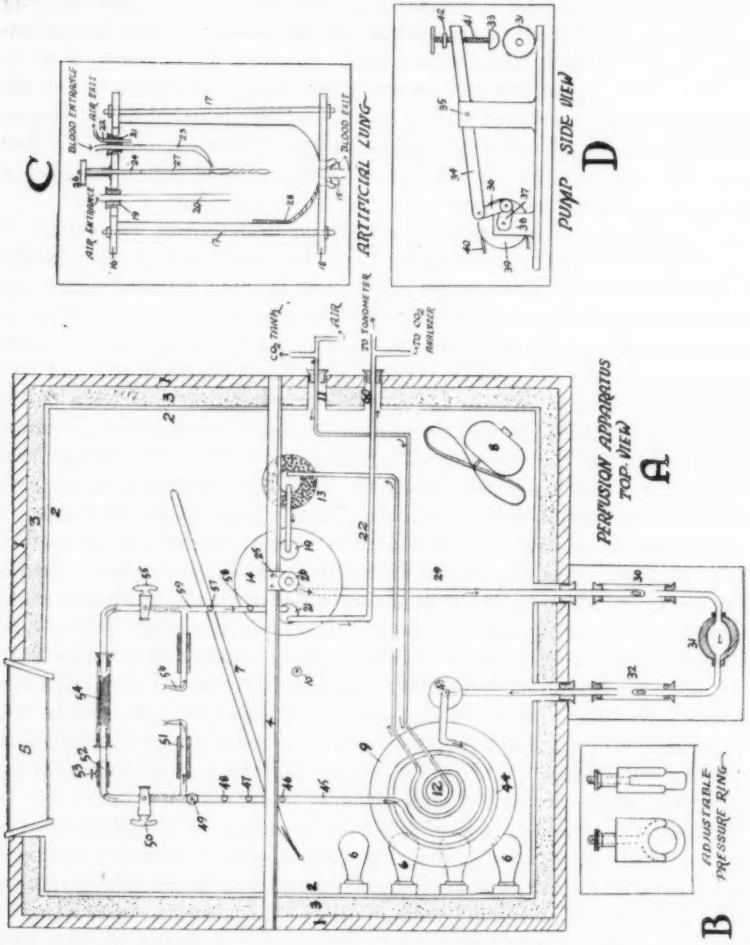

Fig. 1. *A*. Diagram of arrangement of perfusion apparatus, top view.
B. Adjustable pressure ring used in assembling apparatus.
C. Diagram of lateral view of artificial lung.
D. Lateral view of pump.

Moist air chamber. *1*, Outer wooden wall; *2*, inner metal wall; *3*, sawdust packing; *4*, septum in roof of box; *5*, double glass door; *6*, electric bulbs connected with *7*, thermo-regulator (electric connections, relay key, etc., not shown); *8*, fan; *9*, water bath; *10*, thermometer.

Air circuit. *11*, Entrance of air mixture in box; *12*, worm in water bath; *13*, bottle of moist beads; *14*, artificial lung; *15*, rubber bung in neck of bottle; *16*, vulcanite top; *17*, four bolts; *18*, wooden plate; *19*, rubber stopper; *20*, air entrance tube; *21*,

the larger one at the site of this glass door. When the interior of the box once reached 37°C., the fluctuations in the course of an experiment were never more than ± 0.25°C.

The blood was aerated in an artificial lung built on the same principle as the one described by Drinker, Drinker and Lund (1922). The quantity of CO_2 and air admitted to the lung was controlled by needle valves; the mixture was warmed by passing through a copper coil immersed in a water bath, *9*, and saturated with moisture by bubbling through a large column of moist beads, *13*. The artificial lung was conveniently made of an inverted 4-liter bottle, the bottom of which had been cut off, covered by a vulcanite disk and sealed by pressure and paraffin. The arrangement of the lung is shown clearly by figure 1 *C*. The blood was directed onto a grooved vulcanite rod (in the later experiments a disc was substituted) which revolved at a speed of about 1800 revolutions per minute. This was sufficient to spray the blood against the side of the bottle where it drained down to the bottom in a thin film. The vulcanite rod was attached to an air-tight brass bearing, fastened to the septum of the box and the vulcanite cover of the artificial lung. The gas mixture leaving the artificial lung was directed to the CO_2 measuring apparatus. The CO_2 tension was determined by bubbling the gas through a Marriott bicarbonate phenol-red solution. Very little adjustment was necessary to keep a constant CO_2 content in the course of an experiment. No attention was paid to the O_2 content of the mixture as it was always sufficient to allow saturation of the blood.

The blood circuit of the perfusion machine was built entirely of glass and rubber. The rubber connections which joined the various glass tubings were secured by specially designed pressure rings (a diagram of which is shown in fig. 1, *B*) to facilitate the assembling of the apparatus. The blood was kept in circulation by periodically compressing a rubber bulb. The arrangement of this pumping device is shown in figure 1, *D*. The minute output of the pump could be varied within wide limits by shifting the belt from one pulley to another and by changing the screw stem of the wooden plunger, *33*. The blood was propelled in the proper direction by

rubber stopper; *22*, air exit tube; *23*, blood entrance tube; *24–25*, two views of brass bearing arrangement; *26*, small pulley (belt and activating motor not shown); *27*, vulcanite centrifuging rod; *28*, scale; *60*, point of air exit. Path of air current indicated by arrows outside of tubes.

Blood circuit. Path of blood indicated by arrows inside of tubes. *29*, Connection of artificial lung and pump; *30–32*, glass valves; *31*, rubber bulb; *33*, wooden plunger; *41*, screw stem of plunger; *42*, set screw; *34*, lever; *35*, bearing; *36*, connecting rod; *37*, crank; *38*, bearing; *39*, two step pulley; *40*, belt (motor not shown); *43*, rubber "aorta;" *44*, coil in water bath; *45* to *51*, arterial connection; *46–47–48*, openings (connections to U-tube manometer, etc., not shown); *49*, opening for thermometer; *50–51*, three-way valves; *52*, shunt; *53*, screw clamp; *54*, cylinder of glass rods; *56–59*, venous connection; *57–58*, openings (connection for measuring blood flow not shown).

means of two glass valves with rubber dam flaps. The whole arrangement was modified from a similar pump developed in this laboratory for class use. The bulb was filled by the hydrostatic pressure exerted by the reservoir of blood in the large bottle. During the compression of the bulb, blood was forced into a large, vertical, pure gum rubber tube which served as an aorta taking up the excess pressure during "systole" and giving the blood a head of pressure in "diastole." The blood was warmed again by passing through a glass coil placed in a water bath. The arterial side of the circuit had openings which served as an exit when cleaning the artificial circuit, as a means of obtaining blood samples for analysis, as a connection with a mercury U-tube manometer for recording mean blood pressure, and as a means of reading the temperature of the blood supplying the organ. The venous side had three openings which permitted washing out of the apparatus, the injection of blood and acid, and the measurement of the blood flow through the organ. In the early experiments the flow was determined by measuring the amount of blood escaping in a minute from one of these side tubes. In the later experiments a calibrated horizontal tube was introduced (not shown in diagram). When a blood flow measurement was desired, the tube was opened to the air by turning a stopcock which automatically made a contact through a magnet, shutting off the return of blood to the lung. The flow per minute was determined by measuring with a stop watch the time necessary for the tube to fill to the 2 cc. mark. This blood was blown back into the system after the reading.

A short-circuit was provided between the arterial and venous side to allow a circulation of blood independent of the organ. The blood could be sent through the short-circuit or through the organ by proper adjustment of a pair of three-way stopcocks, *50* and *55*. A scale, *28*, graduated in 50 cc. and starting at 250 cc. (which was the capacity of the blood circuit exclusive of the artificial lung) was pasted on the outside of the bottle and used to estimate the volume of perfusion blood. The apparatus, when used with a properly prepared organ and homologous defibrinated *whole* blood, reduplicated the conditions existing in the organ before its removal, as well as a perfusion apparatus can.

Proper precautions against bacterial contamination were taken. The whole system was thoroughly flushed with saline before and after each experiment; after every two or three experiments the apparatus was dissembled and cleaned thoroughly, and between experiments an antiseptic solution, either carbolic acid or 70 per cent alcohol, was introduced.

Defibrinated blood obtained on the day of the experiment from dogs under chloretone or ether anesthesia, and kept on ice while preparing the muscle, was poured into the perfusion apparatus a few minutes before the preparation of the muscle was completed, and thoroughly mixed. Just

before connecting the muscle to the machine, all except 300 to 450 cc. was removed from the machine and used for a control or as a reserve.

2. Preparation of the gastrocnemius and method of connecting for perfusion. The manner of preparing the muscle and the facility of connecting it to the blood circuit is perhaps even more important than the construction of the perfusion apparatus, in determining how far the reaction of the isolated muscle may be regarded as the reactions of a denervated muscle left intact in the body. For this reason the procedure employed is given in some detail.

After reflecting the skin from the mid-thigh to the ankle, the sartorius and the hamstrings were severed near their insertion, especial care being taken when cutting the oral belly of the semimembranosus to avoid injuring the femoral vessels beneath it.

This procedure exposed the femoral vessels, the popliteal space, the gastrocnemius and the crural, peroneal and tibial nerves. The nerves were cut first, then all the branches of the femoral vessels and finally the branches arising from the popliteal artery and vein, care being taken not to tie off any branches supplying the gastrocnemius. The tendon of the gastrocnemius was cut and the entire belly freed. The several veins emerging from the margins of its anterior surface were traced to their connection with the popliteal vein and left intact whenever possible. The two heads of the gastrocnemius were next freed and cut near their insertion, the raw edges being ligated. The separation of the gastrocnemius and its blood supply from the rest of the leg was completed by severing the anterior tibial vessels.

The entire belly of the muscle and the course of the vessels supplying it were carefully inspected and all bleeding points, previously overlooked, ligated with meticulous care. The preparation, still connected with the circulation of the animal, was wrapped in moist cotton and allowed to remain undisturbed until connected with the perfusion apparatus. An absence of reddening of this cotton was taken as evidence of a leak-tight preparation.

When the muscle preparation was ready, glass cannulae were inserted into the femoral vein and artery, the latter quickly connected with a reservoir of warm physiological saline for a short time and the preparation flushed out to obviate intravascular clotting. The vascular connection to the animal was severed, the muscle transferred to the box and connected to the blood circuit. The procedure in connecting the muscle was as follows: The stroke of the pump was greatly decreased, the three-way arterial stopcock, *50*, turned so that blood flowed through the arterial connection, *51*. The arterial cannula was then disconnected from the saline reservoir and quickly joined to the arterial side of the circuit, without allowing air to enter. When a flow was established, the venous side of the

circuit was quickly filled with blood and the venous cannula connected to it. The plunger of the pump was then adjusted until a pressure of 80 to 90 mm. Hg was read in the arterial manometer. The whole procedure, outlined above, occupied about two to three minutes.

3. Procedure of experiments. Readings of the blood volume, blood pressure, blood temperature, blood flow and "expired" CO_2 tension were made when the perfusion was established and repeated from time to time in the course of the experiment. The viability of the muscle was checked several times in the course of the experiment and at the end of the experiment, by its ability to respond to electrical stimulation. The study of blood samples was begun after a preliminary perfusion of $\frac{1}{2}$ to 1 hour. The samples were obtained from the arterial side opening. The volume of blood was read after the first sample was taken and 10 to 20 cc. of 0.085 N HCl, in 0.85 per cent NaCl, per 100 cc. of blood were slowly added to the venous side. It was found essential for a thorough mixing to shunt the blood through the short circuit for five minutes, and augment the volume flow. Once or twice during the course of mixing, the stopcocks were turned and blood shunted through the muscle for $\frac{1}{2}$ minute. This procedure in no way interfered with the viability of the muscle as shown by electric stimulation. At the end of the mixing period the muscle was again put in circuit and the stroke of the pump readjusted so that the blood pressure was at the level existing when the previous samples were taken. Blood samples were again taken immediately and at various intervals later.

The lactic acid was determined by the Clausen method, as modified by Long (1924), and the chlorides, according to Van Slyke's macro-method (1923). The CO_2 combining power was measured, at first with Van Slyke's machine (1921) later with the constant volume modification of Van Slyke and Neill (1924). Van Slyke and Cullen's (1917) technique for exposing whole blood to alveolar CO_2 was followed.

The pH was measured electrometrically with Clark electrodes and type K potentiometer. The method used for equilibrating the blood at the CO_2 tension existing in the "expired" air was the one described by one of us (Banus, 1926). The CO_2 analyzing tube through which air from the lung passed and the one through which bubbled the CO_2 and H_2 mixture for the Clark electrode, were matched with each other and their tensions determined by standard Marriott tubes placed alongside. By manipulating the needle valves of the CO_2 and H_2 tanks, the CO_2 tension could be maintained constant within ± 1 mm. The CO_2 readings were always checked before each sample was measured. The time for establishing equilibrium was greatly shortened and the accuracy of the electrodes enhanced by reducing the blood in a separatory funnel with the CO_2-H_2 mixture before placing it in the chamber of the Clark electrode.

Double determinations of each sample were made simultaneously in two

Clark electrode vessels. No reading was accepted if the E. M. F. difference in the two vessels at equilibrium was greater than 2 millivolts in experiments up to 20, and 1 millivolt in later experiments. The value of the calomel cell was checked against N/10 HCl. In this way the experimental error in the experiments up to no. 20 was less than pH ±0.02, and in the later ones the maximum error was pH ±0.015, and usually less than pH ±0.01.[1]

EXPERIMENTAL RESULTS. *1. Control perfusions.* It is essential first of all to establish as a control the extent of changes, if any, occurring in the blood in the course of the perfusion. The data dealing with this point are given in table 1. There was a slight increase in the lactic acid content and a slight decrease in CO_2 combining power during the first two hours of perfusion of the muscle preparation. The changes in pH and chloride content in this period were within the limits of experimental error (table 1–A). But when the perfusion was continued for 4 hours or more, then noticeable changes in pH and alkaline reserve were observed (cf. expt. 26). These changes were not due to the presence of isolated tissues, but arose from prolonged perfusion in the artificial circuit, as shown by the appearance of similar changes in pH and CO_2 combining power after 4 hours or more circulation in the artificial circuit alone (table 1–B).[2]

The effects of protracted perfusion have been emphasized in order to show *the necessity of obtaining data on acidity changes within a period of 2 or, at most, 3 hours after perfusion is started.* In the present investigation all data obtained after this interval were discarded and our analysis of results is limited to this period of time.

2. The effect of perfusion through isolated muscle on the acidity of blood produced by the addition of hydrochloric acid. A preliminary series of experiments was carried out to compare the effect of adding hydrochloric acid to blood perfused through isolated muscle with its effect on *in vitro* blood.[3] Ten cubic centimeters of acid solution per 100 cc. of blood were added to both samples in three of these experiments and 20 cc. per 100 cc. of blood to the other three. The changes in the perfusion blood, even after 1 to 2 hours' additional perfusion, were the same as in the *in vitro*

[1] Further details of the pH method will be given by Banus (in press, 1927).

[2] We noticed that a darkening of the color of the blood occurred after 4 hours of perfusion. Spectroscopic examination, to our surprise, failed to reveal any methemoglobin formation; nor was there any appreciable hemolysis. The cause of these changes in the blood on protracted perfusion is obscure at present.

[3] These controls were made as follows: Blood was removed from the artificial circuit before connecting the muscle and placed in a horizontal tonometer outside the air bath. This blood was constantly shaken by a weighted string wound around it and attached to the movable lever of a windshield cleaner. The blood was exposed to the air leaving the artificial lung. A bottle of beads was placed in the circuit to absorb the excess moisture.

control. This fact indicates 1, that there is no migration of chloride or any other acid or basic radical between blood and muscle following the addition of hydrochloric acid; and 2, that the perfusion of such blood through isolated muscle does not increase the buffering capacity of the blood.

TABLE 1

Changes occurring in blood during perfusion

EXPERIMENT NUMBER	TIME SAMPLE TAKEN AFTER PERFUSION BEGUN	pH*	CO_2 COMBINING POWER	CHLORIDES	LACTIC ACID
			vol. per cent	*millimols per liter*	*millimols per liter*
A. Through isolated gastrocnemius or hind-leg preparation					
20	1 hour after	7.23		91	1.2
	2 hours after	7.26		90	1.4
25†	Before		34		
	1 hour after		33		
	1½ hours after		33		
26†	Before	7.13	33		
	1 hour after	7.10	31		
	2 hours after	7.10	31		
	4 hours after	7.05	27		
B. Through perfusion apparatus alone					
23	Before	7.40	47		
	1 hour after	7.38	45		
	2 hours after	7.38	44		
	4 hours after	7.37	40		
	6 hours after	7.24	35		
24	Before	7.34	42		
	1 hour after	7.35	42		
	2 hours after	7.34	41		
	4 hours after	7.34	38		
	6 hours after	7.14	28		

All determinations made on whole blood.

* Measured at CO_2 tension of 28 mm.

† Hind-leg preparation.

The validity of these conclusions might be objected to on the ground that the *in vitro* blood was not an adequate control, in view of the fact that the measurement of the volume of the perfusion blood was only accurate within 25 cc. because of the large cross section area of the bottle.

In the later experiments, reported in table 2, the effect of muscle on the acidified blood perfused through it, was determined by comparing samples drawn from the arterial side of the blood circuit immediately after acid was mixed and *before* the perfusion of the muscle was resumed, with samples taken after various periods of perfusion.

Table 2–A includes the data of six experiments in which chloretone anesthesia was used. In all of these experiments, except no. 19, acid was added approximately in the proportion of 20 cc per 100 cc. of blood, i.e., 16.6 per cent of the final volume, producing an increase of about 0.016 N HCl. In experiment 19, 10 cc. per 100 were added, the resulting increase in normality being 0.009 N HCl. The rest of the increase in Cl ions content is due to the Cl of the saline used.

The "alveolar" CO_2 was maintained at a tension of 28 mm. and the pH was determined at the same CO_2 tension. The ratio of perfusion blood to muscle perfused varied from 5 to 1 to 3 to 1, and in the four experiments in which the flow was measured it will be seen that muscle came in contact with the entire blood anywhere from 3 to 6 times.

In none of these experiments was there any evidence, in the course of 1 to $1\frac{3}{4}$ hours of perfusion, of an increase in buffering capacity of the blood. The pH at the end of an hour and $1\frac{3}{4}$ hours was the same within experimental error as it was when the muscle was first perfused with acidified blood. The changes registered were: an increase in pH of 0.01, of 0.02, of 0.03 respectively, in three cases, no change in one case, and a decrease pH of 0.02 in one case.

The changes in lactic acid were inconsistent and of no significance as regards the acid-base balance of the blood. Sometimes there was a slight decrease, as in experiments 15 and 19, in others there was an increase, as in experiment 14, and in still others it remained unchanged (expt. 16, table 2–A).

There was no increase in the CO_2 combining power of the acid blood after perfusion through the muscle. In several experiments in fact (i.e, 19 and 16) there was a diminution of 2 to 3 volumes per cent, which was outside the experimental error; this however also occurred in the control perfusion, as we have already shown above (table 1).

The chloride content also remained unaltered (within experimental error), e.g., in experiment 14, the values obtained immediately and $1\frac{1}{2}$ hours after adding acid were respectively 112 and 113 millimols per liter, etc., showing that there was no migration of chloride into the muscle.

In order to make certain that these negative results were not due to an effect of chloretone on the permeability of the muscle tissue, the experiments were repeated in preparations and with blood obtained from animals anesthetized with ether.

The data of these experiments are given in table 2–B. In all three,

TABLE 2

The effect of perfusion through isolated muscle on the changes produced in the blood by the addition of hydrochloric acid

EXPERIMENT NUMBER	RATIO OF AMOUNT OF BLOOD AFTER ACID ADDED TO AMOUNT OF MUSCLE	NUMBER OF TIMES BLOOD FLOWED THROUGH MUSCLE AFTER ACID ADDED	TIME SAMPLE TAKEN IN RELATION TO TIME ACID ADDED	pH	CO_2 COMBINING POWER	CHLORIDES	LACTIC ACID
			A. Chloretone-morphine anesthesia				
	cc. to gram				*vol. per cent*	*millimols per liter*	*millimols per liter*
19	4 to 1		Before	7.28	33	100	1.0
			Immediately after	6.99	21	111	0.9
		6	1½ hours after	7.00	19	114	1.1
13	4 to 1		Before	7.19	37	97	0.8
			Immediately after	6.71	14	121	0.7
			1 hour after	6.74	15	120	0.8
14	5 to 1		Before		31	89	0.9
			Immediately after		16	112	0.7
			1½ hours after		14	113	1.1
15	5 to 1		Before	7.25	31	101	0.7
			Immediately after	6.70	15	121	0.9
		3	1½ hours after	6.70	13	119	0.6
16	3 to 1		Before	7.14	33	85	0.8
			Immediately after	6.57	18	105	0.7
		4	1¾ hours after	6.55	15	103	0.7
18	4 to 1		Before	7.25	30	87	1.0
			Immediately after	6.89	15	108	0.8
		3	1½ hours after	6.91	16	106	0.9
			B. Ether anesthesia				
33	4 to 1		Before	7.17	33	84	
			Immediately after	6.93	22	96	
		1	1 hour after*	6.92	21	99	
34	5 to 1		Before	7.16	35	84	
			Immediately after	6.86	17	99	
		2	1½ hours after	6.83	16	100	
36	3 to 1		Before	7.36	30	87	
			Immediately after	7.05	21	100	
		9	1⅛ hours after†	7.04	20	99	

All determinations made on whole blood.

* Control acidified blood kept at 37°C. during this period of time, had a pH of 6.91 and a CO_2 combining power of 21 vol. per cent.

† Control acidified blood kept at 37°C. during this period of time, had a pH of 7.05 and a CO_2 combining power of 21 vol. per cent.

20 cc. of acid solution were added to each 100 cc. of perfusion blood. The alveolar CO_2 tension and the CO_2 tension at which the pH was determined were kept constant at 34 mm. Lactic acid determinations were not made in view of the insignificance of the changes in the previous experiments. The CO_2 determinations were made with the Van Slyke and Neill constant volume apparatus (1924).

A glance at table 2–B shows that even under these conditions perfusion through the muscle does not cause an increase in pH or CO_2 combining power, nor does any migration of chlorides occur. The determinations made immediately and at 1 to $1\frac{1}{2}$ hours after the acid was added agree with one another in each experiment, within experimental error. In fact, in all three experiments the very small changes in pH are toward the acid side as are also the changes in CO_2 combining power.

Particular attention is called to the fact that in experiment 36 no change in the acid-base balance of the blood occurred even after the acidified blood was perfused nine times through the muscle.

In experiments 33 and 36 a further control was made to rule out the possibility that an increased acidity of the blood, kept at 37°C., might mask any effect of the muscle. A sample of the acidified blood taken immediately after mixing acid was kept inside the air bath while the rest of the blood was perfused. The pH and CO_2 combining power of this sample were determined at the same time as that of the perfused blood. The changes were found to be the same in both control and perfused bloods (cf. footnote at end of table 2).

Discussion. Before these results can be accepted as applying to muscle in the body, we must determine whether the isolated muscle preparation is in any way comparable with it. In the perfusion system the temperature, pH, blood pressure, blood flow and CO_2 tension of the blood were the same as in the intact animal. The blood used was also similar except for the absence of fibrinogen. The oxygen saturation was equivalent, amounting to 80 or 90 per cent saturation, even at the end of 3 hours' perfusion (Rapport and Katz, 1927).

The conditions under which the muscle was perfused differed from those in the body in that it was denervated and that, in the earlier experiments, a non-volatile anesthetic was used. The results were the same, however, when a volatile anesthetic, ether, was employed later. At two periods in the course of the experiment, each of less than 5 minutes, the circulation through the muscle was interrupted, i.e., while connecting the muscle to the perfusion apparatus and while mixing the acid with the blood. There is no evidence to indicate that the brief anoxemic periods had any influence on the resting muscle, as the subsequent supply of oxygen was large.

The muscle was still responsive to faradic stimulation even after 4 hours of perfusion. The amount of lactic acid in the blood did not appreciably

increase, in any case, after this long period, and in fact in most cases it tended to decrease, e.g., in experiment 13 from 0.08 gram to 0.07 gram per liter after 3 hours, and in experiment 18, from 0.09 gram to 0.05 gram per liter after 3½ hours of perfusion. No appreciable edema was observed in the perfused muscle when the perfusion was not carried on for more than 3 hours, e.g., in experiment 36 the weight of the perfused muscle was 114 grams and the control muscle taken from the opposite leg, 105 grams. Although Rapport and Katz (1927) have shown that in this type of preparation the metabolism is gradually decreasing, to an extent approximating 30 per cent of its original value after 3 hours of perfusion, they have also shown that this is a reversible process. At any point during this period, such physiological stimulants, as for example, glycine, will raise the metabolism of the muscle to a level considerably above the original. We may, therefore, conclude that the preparation used is equivalent to normal denervated muscle in the body.

Hydrogen ion determinations are of prime importance in estimating the effect of muscle tissue on the acid-base balance of acidified blood perfused through it. Under the conditions of the experiments any change in pH towards the original value must indicate an effect of the muscle, as the CO_2 tension was maintained constant. Our results show that there is no measurable change of pH. This is corroborated by the fact that no significant changes in CO_2 combining power or chloride content of the perfused whole blood occur. These results indicate that within the limits of error of our experimental methods, all the acid radicals introduced in the blood remain there and that no basic radicals are contributed to it by the muscle,[4] the buffering capacity of the blood consequently remains unchanged. The effect of the muscle is thus at best insignificant in maintaining the acid-base equilibrium of the blood, as compared to the buffering rôle of the red blood cells, or to the action of the renal and respiratory mechanisms.

Several objections might be raised against the validity of these results. It might be argued that the time of contact of the blood with the muscle was not sufficient; that the amount of muscle used in comparison to the volume of the blood was too small to produce a measurable change; that the effect of the muscle on the blood during the perfusion was of such a nature as to mask the possible effect of the muscle under natural conditions, and that hydrochloric acid is not a physiological acid.

The last objection is not tenable inasmuch as HCl when injected into the blood in the quantities used will combine completely, for practical purposes, with the alkaline reserve of the blood. Thus, we deal in this case, not with an unphysiological presence of HCl, but with an increase of

[4] Theoretically one might consider an exactly balanced equilibrium between such opposite exchanges.

the chloride and the hydrogen ion concentration of the blood and a reduction of its alkaline reserve.

The objection that the blood did not come in contact with the muscle for a sufficient length of time is not valid. According to Austin, *et al.* (1922) complete equilibrium between plasma and blood cells can be obtained in less than 15 minutes. In our experiments the acidified blood was perfused through the muscle for at least 1 hour and in many instances longer. During this time all of the blood passed through the muscle four times on the average, and in one case, nine times. If the rate of exchange between blood and muscle tissue were of the same order of magnitude as that between plasma and red blood cells, there would have been sufficient time to give a measurable indication of an exchange, even though complete equilibrium might not have been reached.

TABLE 3

The effect of adding to acidified blood an amount of buffering material comparable to that contained in the perfused muscle

EXPERIMENT NUMBER		CONTROL BLOOD	ACIDIFIED BLOOD (CONTROL BLOOD PLUS 16.6 VOL. PER CENT ACID SOLUTION)	ACIDIFIED BLOOD PLUS $\frac{1}{2}$ BY VOLUME OF CONTROL BLOOD	EFFECT OF ADDING $\frac{1}{2}$ BY VOLUME OF CONTROL BLOOD TO ACIDIFIED BLOOD
21	pH (at CO_2 tension of 40 mm.)	7.01	6.68	6.75	0.07
	CO_2 combining power in vol. per cent	26	13	17	4
22	pH (at CO_2 tension of 28 mm.)	7.32	6.95	7.03	0.08
	CO_2 combining power in vol. per cent	51	25	30	5

Control perfusion experiments (table 1) show that the effect of the isolated muscle on the blood was not enough, in the period of time of our experiments, to mask any possible increase in the buffering capacity of the acidified blood.

The ratio of muscle to blood in our experiments was 1:5, 1:4 or 1:3, whereas in the whole animal the proportions are approximately 3:1. In other words, the relative amount of muscle used in our experiments was about $\frac{1}{12}$ of that in the whole animal.

A test was devised to determine whether this relatively small amount of muscle was sufficient to produce measurable changes in the blood. Control blood instead of perfused muscle tissue was added to the acidified blood in a proportion equivalent to that existing between muscle and acidified blood in our experiments. This procedure was justified as it has been

shown that the buffering power of muscle tissue is at least equal to that of the blood (Katz, Kerridge and Long, 1925). The minimal proportion of 1:5 was used in making the test. The results, which are shown in table 3, indicate clearly that the amounts of buffering material present in the muscle are sufficient to produce measurable changes in the hydrogen ion concentration and alkaline reserve of the blood if they could come in contact with it. None of these objections is, therefore, valid and consequently our results can only be explained as due to the fact that the isolated muscle has no effect, at least none that is measurable, on the acid-base balance of the acidified blood perfused through it.

The fact that the buffering material in the muscle is not available as a reserve for the blood, tends to indicate a low degree of permeability in the muscle tissue to the excess of Cl and H ions as well as to any cations which might compensate for the decreased alkaline reserve of the blood.[5] This low degree of permeability is in our opinion corroborating evidence in favor of the physiological condition of the muscle during the experiment, for it is well established that the permeability of living tissue increases as death is approximated and that at death its *semipermeability* disappears (Osterhout, 1922).

SUMMARY

1. An apparatus for perfusing isolated organs with fully oxygenated, non-acapnic, defibrinated or heparinized whole blood, which provides an adequate control of the pressure, flow, temperature and CO_2 tension of the blood, is described.

2. A method is described for isolating the gastrocnemius muscle of the dog, and for perfusing it under conditions as physiological as possible.

3. Reasons are given for assuming that the results obtained on such a denervated perfused muscle preparation are applicable to muscle tissue in the body, provided the perfusion is limited to a period not exceeding 3 hours.

4. The availability of the buffering material in the muscle as a reserve for the blood was determined by perfusing the gastrocnemius with blood whose hydrogen ion concentration had been changed, within physiological limits, by the addition of dilute hydrochloric acid.

5. The changes in pH, CO_2 combining power and chloride content of the whole blood were determined, at constant CO_2 tensions. The pH determinations were made by a modification of the electrometrical method.

6. The results obtained in perfusing the gastrocnemius of the dog indicate that no measurable exchange of chloride or alkaline radicals occurs

[5] This statement, of course, does not preclude the possibility of an increase in permeability during increased activity.

between the blood and the muscle, following perfusion of the latter with blood acidified with HCl, and consequently the acid-base balance of the blood is unchanged.

7. When the CO_2 tension is maintained constant, muscle tissue does not increase the buffering capacity of the blood within measurable limits.

8. The buffering materials which are known to exist in the muscle in an amount sufficient to produce a measurable change in the acid-base equilibrium of the blood, if they could come in contact with it, are not available to the blood under the conditions of the experiments. This seems to indicate a lack of permeability of the muscle tissue, preventing the diffusion of the chloride radical from the blood to the muscle as well as that of the buffering material of the muscle into the blood.

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OBSERVATIONS ON THE RÔLE OF TISSUES IN MAINTAINING THE ACID-BASE EQUILIBRIUM OF THE BLOOD

II. Effect of Hind-Leg Preparation

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In a previous paper (Katz and Banus, 1927) it has been shown that isolated muscle has no effect on the acid-base equilibrium of acidified blood perfused through it. The chloride ions added to the blood in acidifying it with hydrochloric acid, remain in it and no other radicals capable of modifying the alkaline reserve of the blood are added by the muscle. While these facts apply to isolated muscle tissue, it still remains to be seen whether or not other tissues have any influence on the acid-base equilibrium of the acidified blood perfused through them.

Atzler and Lehmann (1922) have shown that surviving mammalian hind-leg preparations increase the pH of acid-saline perfused through them. Several objections to their results have already been raised in our previous communication, the most important being based on the low buffering capacity and anoxemic condition of the perfusate. There is no evidence that the permeability of the tissues was not increased under the anoxemic conditions. In view of this fact and because of the results obtained by us on isolated muscle tissue, we deemed it advisable to repeat the perfusion of the isolated hind-leg with acidified whole blood, under conditions which were more nearly physiological.

Recent work by Gesell and Hertzman (1926) has shown that the changes in pH, following alterations in the CO_2 or bicarbonate content of the blood, are not simultaneous in the carotid artery and jugular vein. The delay in the change of pH in the venous blood is ascribed by them to the buffering activities of the tissues. Inasmuch as their pH determinations were not made at a constant CO_2 tension, one cannot conclude from their results that this buffering activity of the tissues is due to influences other than a migration of CO_2. The fact that an excess of CO_2 in the blood will migrate into the tissues has recently been reëmphasized by Shaw (1926) and by Rapport and Katz (1927).

The same objection applies also to the results of Rous, Drury and

Beattie (1927), who showed by intra-vital stains that the injection of HCl into the blood stream decreased the pH of some tissues, notably connective tissue. Their results, furthermore, did not give any direct evidence of the possible effect of the tissues on the acid-base balance of the blood.

In the present investigation, as in the previous one, the effect of changes in CO_2 tension was eliminated by keeping this tension constant during the entire experiment and by measuring the pH and alkaline reserve at constant CO_2 tensions. This was done in order to determine whether or not the tissues in the hind-leg have any buffering effect on the blood other than that of allowing CO_2 to diffuse into them.

The hind-leg of the dog was perfused with whole defibrinated blood by means of the perfusion apparatus, previously described by us (1927), which, as has been pointed out, is well suited for such an analysis. The technique of the experiment was the same as in our previous paper (1927). The method of preparing the hind-leg for perfusion has already been described by Rapport and Katz (1927). Ether anesthesia was employed both for the operation and for procuring the blood. The CO_2 tension of the blood was maintained constant at 34 mm. Hg during the perfusion, and the pH measurements were made at the same tension. The CO_2 combining power was determined with Van Slyke and Neill's (1924) constant volume apparatus. The lactic acid changes were not measured, as the previous investigation had shown that they are of no significance under the experimental conditions. In all cases 20 cc. of 0.085 N HCl in 0.85 per cent NaCl were added to each 100 cc. of perfusion blood. The duration of perfusion was never carried beyond three hours.

EXPERIMENTAL RESULTS. The results of four such experiments are shown in table 1. The ratio of muscle to blood varied from 4:1 to 2:1 and the ratio of blood to total tissues perfused was 1:1½ and 1:2 in the two experiments in which the tissues were weighed. Calculations showed that in the hour period, blood was in contact with the tissues anywhere from 6 to 12 times.

When the pH readings of the sample taken after one hour's perfusion were compared with the control sample taken immediately after the acid had been thoroughly mixed with the blood, the pH was found to have increased in every instance; the increase in pH being 0.07 in experiment 29, 0.19 in experiment 30, 0.08 in experiment 31 and 0.29 in experiment 37. In experiment 31 another hour's perfusion increased the difference in pH to 0.11.

The CO_2 combining power of the sample, taken after an hour's perfusion, showed a striking increase in experiments 30, 31 and 37 over that of the sample obtained immediately after mixing the acid with the blood; the increase amounted to 2, 5 and 7 volumes per cent, respectively, in

these experiments. In experiment 29 no change in CO_2 combining power was observed. The negative result in this experiment can be discounted in view of the marked change in the other three experiments.

The chloride content of the blood samples, taken one hour after perfusion with acidified blood, was the same, within the limits of experimental error, as the sample taken immediately after the acid had been mixed with the blood. In experiment 31 an additional hour's perfusion

TABLE 1

The effect of perfusion through the hind-leg preparation on the changes produced in the blood by the addition of hydrochloric acid

EXPERIMENT NUMBER	RATIO OF AMOUNT OF BLOOD AFTER ADDITION OF ACID, TO AMOUNT OF MUSCLE	RATIO OF AMOUNT OF BLOOD AFTER ADDITION OF ACID, TO AMOUNT OF TISSUE IN PREPARATION	NUMBER OF TIMES BLOOD FLOWED THROUGH TISSUES AFTER ACID ADDED	TIME SAMPLE TAKEN IN RELATION TO TIME ACID ADDED	pH	CO_2 COMBINING POWER	CHLORIDES
	cc. to gram	*cc. to gram*				*vol. per cent*	*millimols per liter*
29	4 to 1			Before	7.04	28	95
				Immediately after	6.78	21	111
			6	1 hour after	6.87	21	108
30	4 to 1			Before	7.08	30	106
				Immediately after	6.63	21	122
			10	1 hour after	6.82	23	123
31	3 to 1	1 to 1.5		Before	7.08	26	90
				Immediately after	6.79	17	108
			7	1 hour after	6.87	22	108
			14	2 hours after	6.90		108
37	2 to 1	1 to 2		Before	7.09	31	89
				Immediately after	6.54	12	107
			12	1 hour after	6.83	19	106

All determinations made on whole blood.

left the chloride content still unchanged; in the three samples of this experiment it remained at 108 millimols per liter.

The hind-leg preparation, in striking contrast to isolated muscle, has a definite effect on the acid-base balance of acidified blood perfused through it. This is clearly shown in figure 1. The figure illustrates the changes in pH, CO_2 combining power, and chloride content of acidified whole blood, following perfusion through the hind-leg preparation, as compared with those produced by perfusion through isolated muscle. For the sake of simplicity, two typical experiments on each of these

preparations are shown. The solid line curves are from experiments on isolated muscle, the dotted line curves are from hind-leg perfusions. In addition, a control perfusion without the addition of acid, is illustrated by the dash line curve. In each curve the values of pH, etc., of the control blood, before the addition of acid, is taken as zero, so that the

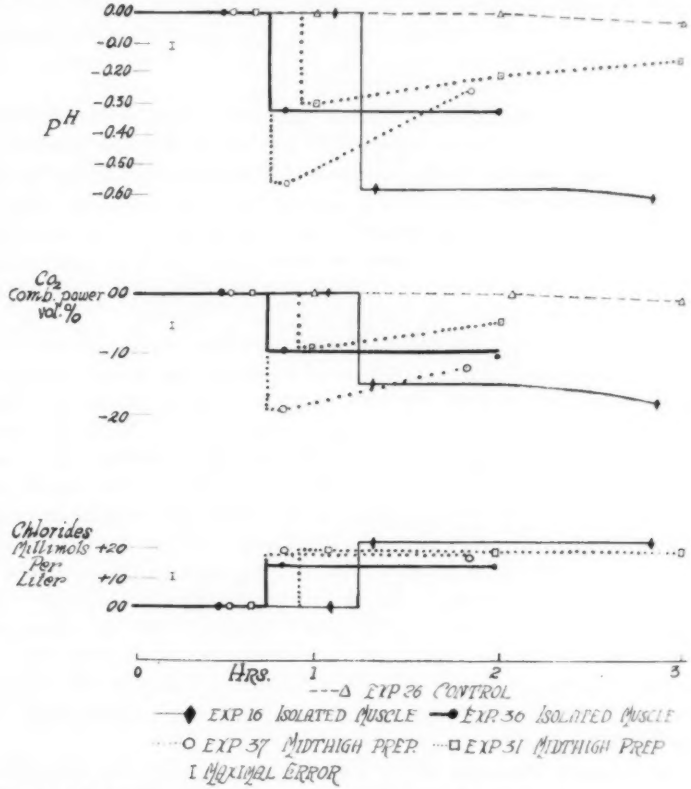

Fig. 1. Graph showing the typical changes in pH, CO_2 combining power, and chloride content of blood, following perfusion through isolated muscle or hind-leg preparations. Described in text.

ordinates show the deviations from these values during perfusion. The abscissae give time in hours from the beginning of perfusion. The vertical part of each curve shows the sudden change produced by the addition of acid and the portion of the curves after this time indicates the effect of the preparation on the perfused acidified blood.

A glance at this figure will show that, as previously reported (1927), no

change occurs in the pH or CO_2 combining power of the blood when perfused through the isolated muscle; the curves remaining parallel to the base line except toward the end of the three hours' perfusion when a slight drop in the curve can be noticed. This drop is practically parallel to that present in the control perfusion at this time. In the case of perfusion through the hind-leg preparation, the curves show a decided tendency to return to the values of pH and CO_2 combining power present in the unacidified control blood. In both types of preparation, the chloride curve remains parallel to the base line.

The last fact is conclusive evidence that the effect of the hind-leg preparation is not due to a migration of the excess chloride ion out of the blood. The inability of the tissues in this preparation to take up the excess chloride ion from the blood is confirming evidence of the relative impermeability of tissues in general—other than the red blood cell—to the chloride ion. The literature on this point has been adequately summarized by Smith (1926) and since then further evidence has been brought forward by Michaelis (1926) and Lillie (1926).

It follows, therefore, that the alkali reserve bound by the excess Cl is not set free and the increase in pH and CO_2 combining power, following perfusion, must be explained on other grounds. Three possibilities may be considered; 1, a shift of acid radicals, other than Cl, from the blood to the tissues; 2, a shift of alkaline radicals from the tissues into the blood, and 3, a possible increase in the total amount of buffers in the blood, e.g. produced by an increase in the number of red blood cells circulating in the blood as a consequence of bone marrow activity. There is not sufficient evidence at present to evaluate which of these mechanisms operates to produce the resultant change in the buffering capacity of the blood. The possibility of a migration of CO_2, the most diffusible of all the other acid radicals which might be considered, has been eliminated from our experiments. Lactic acid migration may be disregarded, as the amount present in the blood was insufficient to account for the magnitude of the change in blood acidity.

It also remains unsettled which tissues (bone, marrow or connective) exchange radicals with the blood in our preparation. Our previous results, however, apparently eliminate muscle tissue.

SUMMARY

1. The isolated hind-leg of the dog was perfused with whole blood, acidified with dilute HCl. The technique employed was the same as that described in a previous report.

2. An increase in pH and CO_2 combining power of the acidified blood was observed following perfusion. No alteration in the concentration of the Cl ion of the whole blood occurred.

3. These results indicate that some of the tissues in the hind-leg have an effect on the acid-base equilibrium of the blood in the sense that they increase the buffering capacity of the blood perfused through them.

4. This influence is not due, according to our previous results, to the muscles contained in the preparation. It is not due to a migration of Cl nor of CO_2 out of the blood. The exact nature of the processes, as well as the tissues involved, is at present unknown.

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THE EFFECT ON THE CIRCULATION IN MAN OF REBREATHING DIFFERENT CONCENTRATIONS OF CARBON DIOXIDE

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The effect of carbon dioxide upon the circulation has been studied extensively in laboratory animals; in the human subject observations have been less numerous. The present paper aims to present a quantitative analysis of the more prominent effects in man.

METHOD. The subject rebreathed from a Douglas bag containing approximately 50 liters of the gas mixture. The arterial blood pressures were read by the auscultatory method, using a mercury manometer. Respiratory rate and amplitude were recorded on a kymograph, using a rubber tube around the chest which was connected to a Marey tambour. The heart rate was counted at the radial artery; no graphical records were taken. Alveolar air samples were taken directly into a Henderson gas analyzer. Samples were taken as frequently as they could be analyzed; the average time required for one carbon dioxide analysis was two minutes.

Normals were read in each experiment just before the rebreathing started; and the effects were followed for several minutes after the rebreathing had ended.

The mixture to be rebreathed was made up in the Douglas bag by running in carbon dioxide from a gas generator and then diluting with the calculated volume of room air. The subject continued to rebreathe as long as possible without inviting too unpleasant effects. In many instances the alveolar carbon dioxide tension at the end of the experiment was found to be above 9 per cent and in one case 10 per cent. To stop rebreathing the mouthpiece was taken from the subject, who then remained seated quietly while the after-effects were being recorded. Analyses of the carbon dioxide in the bag were made before and after rebreathing, and the values at intermediate times were interpolated as directly proportional to the intervening time. The work of Hough (1911) and of Lundsgaard and Schierbeck (1923) demonstrated the correctness of this proportionality. From the graphs can be found the concentration of carbon dioxide in the Douglas bag at each stage of the experiment (figs. 1 and 2).

EXPERIMENTS ON REBREATHING INCREASING CONCENTRATIONS OF CARBON DIOXIDE. 1. *Alveolar carbon dioxide tension.* Regardless of the initial carbon dioxide concentration of the inspired air (provided only that it is less than 5 per cent) there is, first, a rise of approximately 1.4 per cent in the alveolar carbon dioxide tension, which occurs within 2 minutes after the start of the experiment (table 1 and fig. 3). At this point the respiratory rate or amplitude or both are increased (figs. 1 and 2), causing a retardation of the initial rise or even a fall in the alveolar tension; which now remains nearly constant until the carbon dioxide tension in the Douglas bag reaches 5 to 6 per cent. The duration of this period is dependent upon the initial concentration of carbon dioxide in the inspired air; a smaller initial concentration requiring a longer time to reach 5 to 6 per cent. When this percentage prevails in the inspired air even the maximal efforts of the respiratory apparatus are ineffective in keeping down the alveolar tension, which rises in relation to but not parallel with the bag tension (table 2). At the end of the experiment the two values are very nearly equal. The alveolar tension followed the above course in all of the experiments in which it was measured.

2. *Systolic blood pressure.* The systolic blood pressure was recorded in 33 experiments in which the initial concentration of carbon dioxide in the bag ranged from 0.2 to 6.6 per cent. In every case the rise in pressure occurred in two definite and discrete stages separated by a period of steady state (figs. 1 and 2). As shown in figure 4, the amount of the initial rise is approximately the same regardless of the starting percentage of carbon dioxide in the Douglas bag; whereas the length of time before this rise ceases is almost directly proportional to the starting percentage. When however the starting percentage is 5 per cent or higher, the blood pressure completes its rise very rapidly; usually in less than four minutes, and the three separate stages are not evident. The duration of the steady state is also proportional to the starting percentage of carbon dioxide. In the final stage the picture is somewhat different. This stage in every experiment begins when the concentration in the bag has reached approximately 6 per cent; and in every case when past this point the blood pressure rapidly rises and shows a characteristic type of curve. The extent of the systolic blood pressure rise in this stage depends only on the ability of the subject to continue to rebreathe, in many instances going 60 mm. of mercury higher than the resting value.

The direct relationship between the rise in blood pressure and the alveolar carbon dioxide tension is very striking. In figures 5 and 6 each point represents a one minute interval and R_1, R_2 and R_3 represent the three stages described above.

In R_1 there are considerable changes in the alveolar carbon dioxide tension and in the systolic blood pressure over a short period of time (1 to 2 minutes).

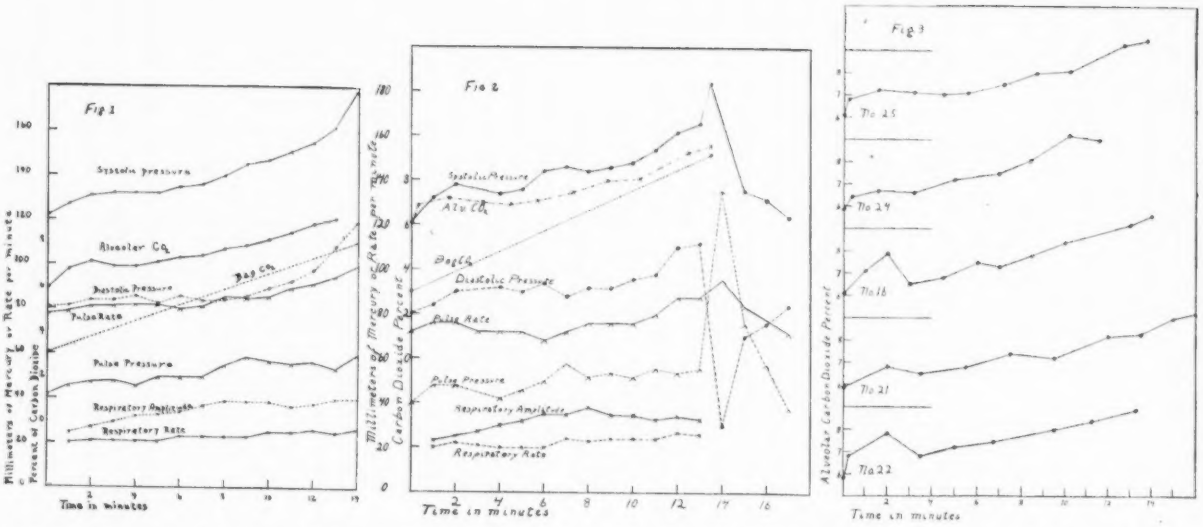

Fig. 1. Time course, average of five experiments (nos. 13, 15, 22, 24 and 25). Respiratory amplitude is given in arbitrary units.

Fig. 2. Time course of a single experiment (no. 25) showing recovery. The diastolic pressure did not usually fall as low as in this particular experiment.

Fig. 3. Time course of changes in alveolar carbon dioxide in five experiments. The initial percentages in the inspired air were: no. 25, 3.0; no. 24, 4.0; no. 16, 3.1; no. 21, 0.2; no. 22, 2.3.

In R_2 there are lesser changes in the alveolar carbon dioxide tension and in the systolic blood pressure over a much longer period of time (5 to 8 minutes).

In R_3 there are very great changes in the alveolar carbon dioxide tension and in the systolic blood pressure in the same or even less time than in R_2.

TABLE 1

Relation of initial carbon dioxide percentages in inspired air to the stages in the increase of carbon dioxide percentages in the alveoli

EXPERIMENT NUMBER	(1) INITIAL CO_2 IN INSPIRED AIR	(2) NORMAL ALVEOLAR CO_2 TENSION	(3) WITHIN TWO MINUTES RISES TO	(4) INCREASE	(5) WITHIN 3½ MINUTES FALLS TO	(6) FINAL BAG CO_2	(7) FINAL ALVEOLAR CO_2 TENSION	(8) DURATION OF EXPERIMENT
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*minutes*
21	0.2	5.7	6.8	1.1	6.5	8.6	9.2	16
22	2.3	5.7	7.8	2.1	6.8	9.0	8.9	14
23	3.6	5.7	7.2	1.5	7.5*	9.2	9.9	11
24	4.0	5.7	6.7	1.0	6.6	8.0	9.0	12
25	3.0	6.1	7.2	1.1	7.0	9.2	9.5	13

* Increased by 0.3 per cent—no fall.

TABLE 2

Relation between alveolar carbon dioxide and inspired carbon dioxide at different periods in experiment

EXPERIMENT NUMBER	TIME	BAG CO_2	ALVEOLAR CO_2	DIFFERENCE
	minutes	*per cent*	*per cent*	*per cent*
25	1	3.5	6.8	3.3
	6	5.8	7.1	1.3
	10	7.8	8.1	0.3
	13	9.2	9.3	0.1
17	1	0.7	6.3	5.6
	6	2.9	6.6	3.7
	10	4.7	7.3	3.1
	14	6.5	7.0	0.5
	15	6.9	7.2	0.3

This similarity in the time relations of the alveolar carbon dioxide tension and the systolic blood pressure is also shown by the very similar curves given by each in figures 1 and 2. Table 3 summarizes these data and also emphasizes the fact that in rebreathing by normal subjects such as were used in these experiments, the final stage in the blood pressure rise, which very quickly forces the subject to end the experiment begins only when the carbon dioxide concentration in the bag is approximately 6 per cent and the alveolar tension 7.2 per cent.

3. *Diastolic blood pressure.* For five experiments ending with 9 per cent of carbon dioxide in the bag, the average maximal increase in diastolic

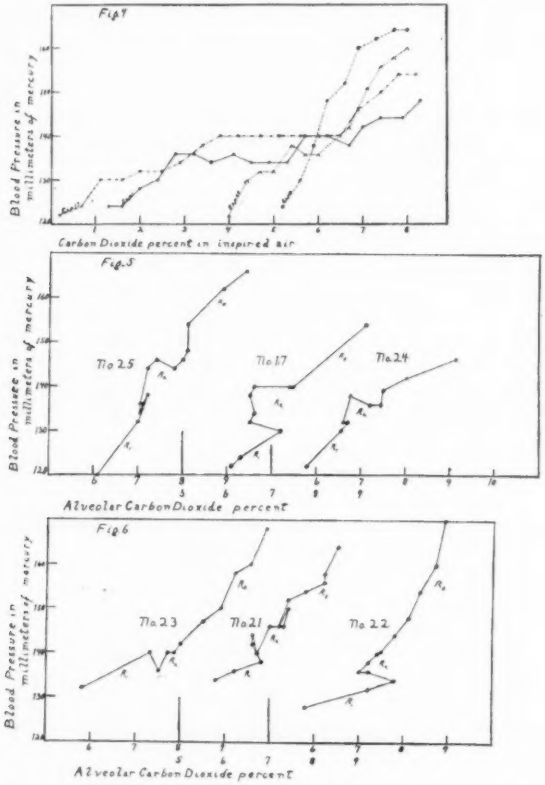

Fig. 4. Relation of systolic blood pressure to the carbon dioxide concentrations in the inspired air in four experiments. The carbon dioxide was calculated in each case from the two analyses of the carbon dioxide in the bag which were made before and after the rebreathing (see *Bag* CO_2 in figs. 1 and 2).

Fig. 5. Relation of blood pressure to alveolar carbon dioxide. The points are connected as they occurred consecutively at one minute intervals in the experiments. R_1, R_2 and R_3 indicate the three stages discussed in the text.

Fig. 6. Relation of blood pressure to alveolar carbon dioxide. The points are connected as they occurred consecutively at one minute intervals in the experiment. R_1, R_2 and R_3 indicate the three stages discussed in the text.

pressure was 22 mm. of mercury. The rise in diastolic pressure shows the same relationship to the alveolar carbon dioxide tension as does the systolic pressure. The one difference, as shown in figure 7A, is that the initial rise

in diastolic pressure is slower than in the systolic pressure; so that in R_1 while the alveolar carbon dioxide concentration increases 1.4 per cent in approximately one minute, the diastolic pressure increases but 1 to 2 mm. of mercury.

At this early stage there is then an increased pulse pressure.

4. *Heart rate.* The averaged increase in heart rate taken for eight experiments ending with bag carbon dioxide tension of 9 per cent was 18 beats per minute above the normal. When plotted against time or the alveolar carbon dioxide tension, the heart rate gives a curve similar to those found by plotting these values against the systolic or diastolic blood pressures. Stages R_1, R_2 and R_3 are shown in figure 9B.

TABLE 3

Relation of the stages in the blood pressure rise to the alveolar carbon dioxide tension and the carbon dioxide in the inspired air

EXPERIMENT NUMBER	(2) INITIAL CO_2 IN INSPIRED AIR	(3) NORMAL SYSTOLIC BLOOD PRESSURE	(4) SYSTOLIC PRESSURE AFTER		(5) TIME BEFORE SUDDEN RISE	(6) BAG CO_2 AT SUDDEN RISE	ALVEOLAR CO_2 AT SUDDEN RISE
			One minute	Two minutes			
	per cent	*mm.* Hg	*mm.*	*mm.*	*minutes*	*per cent*	*per cent*
1	1.3	124	124	128	10	5.3	
2	5.2	124	130	138	2	5.6	
3	2.5	126	128	132	7	5.9	
4	3.3	120	120	124	6	5.7	
6	2.5	118	122		5	5.6	
7	0.2	118	118	122	11	5.2	7.4
20	0.9	120	128	128	10	5.7	7.2
21	0.2	134	136	138	9	4.9	7.1
22	2.3	128	132	134	7	6.1	7.5
24	4.0	122	130	132	3	6.1	6.9
25	3.0	120	132	138	5	5.3	7.0

5. *Minute volume output of the heart.* The determination of the minute output of the heart in the human subject by any direct or any reliable indirect method is as yet something not realized. Wiggers (1923) and Rosen and White (1926) both support the approximation that the product of the pulse pressure and the pulse rate is an indication of the minute output of the heart. On the basis of this qualitative relationship were calculated the values which are graphed in figure 8. It is interesting to note that the curve in figure 8B is very similar to the curves found by plotting either heart rate, blood pressure, or the alveolar carbon dioxide tension against time.

EXPERIMENTS WITH LOWERED ALVEOLAR CARBON DIOXIDE TENSION. To find the effects of lowered alveolar carbon dioxide tension a number of

forced breathing experiments were performed (fig. 7). In experiment 18, the systolic blood pressure fell 20 mm. of mercury while the alveolar carbon dioxide tension was decreased from 5.7 to 3.4 per cent, in three and one-half minutes of very strenuous forced breathing. Experiment 19 shows

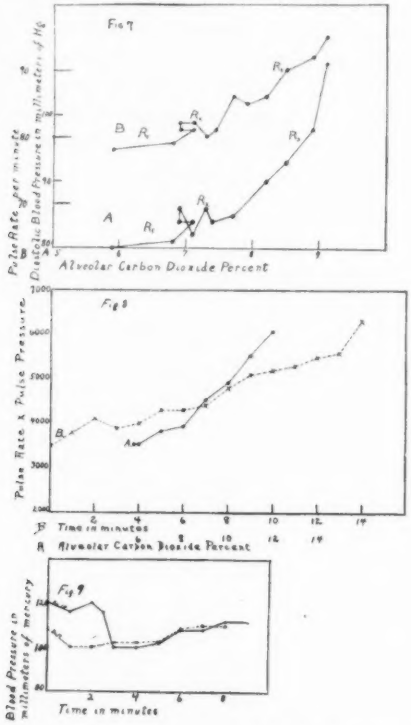

Fig. 7. Relation of diastolic blood pressure, *A*, and of pulse rate, *B*, to alveolar carbon dioxide tension. Average of five experiments, nos. 13, 15, 22, 24 and 25. Each point represents the value one minute after the previous point.

Fig. 8. Relation of the product of pulse rate and pulse pressure to alveolar carbon dioxide tension, A, and to time B. Average of five experiments, nos. 21, 22, 23, 24 and 25.

Fig. 9. Time course of two experiments showing the effect of forced breathing on systolic blood pressure.

Experiment 18—Forced breathing for the first three minutes.

Experiment 19—Forced breathing for the first minute and thirty seconds.

that almost no effect is produced by a lesser amount of forced breathing. In Henderson's (1918) forced breathing experiments the alveolar carbon dioxide was not measured and the systolic blood pressure was reported as not markedly affected.

THE INFLUENCE OF OXYGEN. To find whether the circulatory changes noted were influenced by an increase in alveolar carbon dioxide tension or by a decrease in oxygen tension, a number of experiments were performed using high and low percentages of oxygen in the rebreathed mixture. With the high percentages of carbon dioxide in the bag, the results were the same as those when room air was used to dilute the carbon dioxide. In two experiments in which the final carbon dioxide tensions were 9.7 and 9.0 per cent, the results were similar to those obtained with both high oxygen and room air used in making up the mixture. When however the oxygen in the bag went as low as 6.9 per cent the blood pressure rose very quickly and the subject could not rebreathe for more than three to four minutes. The fact that the oxygen tension does not affect the circulation until it falls below 7 per cent was also noted by Schneider and Truesdell (1924).

TABLE 4

Diastolic blood pressure during the recovery period

NORMAL DIASTOLIC PRESSURE	PRESSURE IN LAST MINUTE OF BREATHNG CARBON DIOXIDE	DIASTOLIC PRESSURE AFTER BREATHING AIR		
		One-half minute	One minute	Two minutes
mm. Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg
84	100	50	70	72
78	84	30	58	
74	102		60	70
76	106	50	70	72
80	110	8	70	76
76	106	50	70	72
74	102	54	70	

RECOVERY PERIOD. After the rebreathing period was ended the observations on the systolic pressure, diastolic pressure and heart rate were continued until the subject had returned nearly to normal.

1. *Systolic blood pressure.* The systolic blood pressure averaged at the end of 15 experiments was 159 mm. of mercury. One minute later it had fallen to 139 mm.; two minutes later to 126 mm.; and so gradually to the normal in from four to six minutes.

2. *Diastolic blood pressure.* The diastolic pressure presents a far more complicated series of events. At the end of the rebreathing period, the diastolic pressure was usually between 100 and 110 mm. of mercury. As soon as the subject was allowed to breathe room air, instead of paralleling the systolic pressure and gradually falling to normal, the diastolic fell very suddenly below the normal level; and then immediately rose again to within 10 to 15 mm. of the normal. As shown in table 4, this rapid fall and equally rapid rise took place within one minute after the rebreathing ended.

3. *Heart rate.* Accompanying this very marked fall in the diastolic pressure there was always a very pronounced increase in the heart rate; in many instances there were increases of 25 to 35 beats per minute in the first minute after the subject was allowed to breathe room air (table 5). The fall in heart rate to the normal level, following this first increase, is not as precipitous as the movements of the diastolic pressure; it usually was accomplished within two minutes.

DISCUSSION. The fact that carbon dioxide produces a number of important effects on the human circulation has been noted for a long time. Hill and Flack (1908) reported that the blood pressure changes previously noted in laboratory animals could be reproduced in man. As far as we could determine, however, Schneider and Truesdell (1922) were the first to undertake an extensive study of this problem with the human subject. In general their results and ours are in accord as to the phenomena occurring during the rebreathing period. These authors however have not emphasized the very rapid and transient changes which occur immediately after the rebreathing period has ended; possibly because their readings were taken at the end of the first minute, at which time the magnitude of the changes would not be apparent.

TABLE 5

Heart rate during the recovery period

TIME	BEATS PER MINUTE EXPERIMENT	
	No. 21	No. 22
Normal	76	76
End of rebreathing	88	88
After 1 minute	120	126
After 2 minutes	104	92
After 3 minutes	96	84
After 4 minutes	84	80
After 5 minutes	76	76

The question as to the nature of the mechanism controlling the blood pressure changes recorded, is well reviewed by Schneider and Truesdell (1922). Qualitative investigations have been reported by Bayliss (1901), Hooker (1911), and Fleisch (1918) on the direct effect of carbon dioxide on the vascular and cardiac tissue; by Kaya and Starling (1910), Mathison (1910 and 1911), Itami (1912), and Dale and Evans (1922) on the action of carbon dioxide upon the vasomotor centers in the medulla; by Jerusalem and Starling (1910), Itami (1912), Patterson (1914), and Schneider and Truesdell (1922) on the changes in the minute volume output of the heart; by Henderson and Harvey (1918), on the influence of venous pressure

changes; and by Anrep (1912a and b), Elliot (1912), Cannon and Hoskins (1912), and Cannon and Rapport (1921) on the influence of the stimulation of the suprarenal glands.

Many varied results and conclusions have been arrived at, so that at present our own feeling is that probably all these factors, and perhaps others, are to a greater or less degree responsible for the blood pressure changes; certainly however additional work is necessary before the extent to which any one factor is concerned can be determined.

SUMMARY

1. Measurements were taken of the systolic and diastolic blood pressures, the heart rate, the alveolar carbon dioxide tension, and the respiratory rate and amplitude, while the subject was rebreathing different concentrations of carbon dioxide.

2. The increase in the systolic blood pressure occurred in three definite stages; the duration of each stage could be regulated by changes in the carbon dioxide concentration of the rebreathing mixture.

3. The systolic pressure, the diastolic pressure and the heart rate showed a characteristic relationship to the changes in the alveolar carbon dioxide tension.

4. During the recovery period, the systolic pressure gradually fell to the normal level, while the diastolic pressure fell very markedly immediately after the subject was allowed to breathe room air and then just as suddenly rose. This transient effect took place within the first minute after the rebreathing period had ended.

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FURTHER ATTEMPTS TO INCREASE EXPERIMENTALLY THE HORMONE OUTPUT BY THE THYROID GLAND

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By methods described in previous publications (1), (2), (3) attempts were made to increase temporarily the concentration of thyroglobulin in the thyroid vein blood in dogs under light barbital anesthesia by stimulation of the peripheral end of the cervical sympathetic nerves, by intravenous injections of adrenalin and pilocarpine, and by direct massage of the thyroid glands. We have reported that by using serum from rabbits injected with purified dog thyroglobulin, the precipitin test reveals the presence of thyroglobulin in lymph from the thyroid gland, in the lymph from the common neck lymphatics, and in the blood of the thyroid vein, but not in the blood from the general circulation, the thoracic lymph or lymph from the legs (2). Attempts to increase the thyroglobulin concentration in the thyroid lymph by nerve stimulation, sympathomimic drugs and massage yielded essentially negative results (3). These results have recently been confirmed by Hicks (4).

We attempted to select dogs with thyroid glands as nearly normal as possible, as judged by direct inspection. None of the thyroids were markedly hyperplastic. Barbital was used, as this anesthetic in dogs seems to induce less organ depression than ether. The thyroid veins were isolated with the least possible trauma to the thyroid glands, for securing the 2 to 5 cc. blood samples. The cervical sympathetic nerves were separated from the vagi and severed low in the neck prior to drawing the initial thyroid vein blood samples, so as to have this condition common to all the experimental factors.

The cervical sympathetic nerves were stimulated with a weak tetanizing current during each alternate minute for one-half hour. The effectiveness of the stimulation was judged by the response of the pupil. The massage of the thyroid gland was done by moderately vigorous hand movements over the skin. The massage period was 30 minutes. The adrenalin and pilocarpine were injected into the femoral vein, adrenalin in quantities of 1 to 5 cc. of a 1–20,000 solution, pilocarpine in quantities of 2 to 20 mgm. The thyroid vein samples were taken ten minutes after the drug injections.

CONTROL TESTS. In two dogs (33 and 34) the following control tests were run under barbital, with the cervical sympathetic nerves severed, and the thyroid veins sufficiently isolated for drawing the blood samples, and blood samples drawn every 30 minutes:

Dog 33 { Left thyroid vein blood: 4, 8, 4, 2, 4, 4
Right thyroid vein blood: 4, 8, 8, 8, 4, 4

Dog 34 { Left thyroid vein blood: 8, 8, 4, 8, 8
Right thyroid vein blood: 4, 4, 4, 4, 4

The figures indicate the maximum dilution of the thyroid vein serum that yielded a definite precipitin test with the rabbit antiserum. These tests indicate a considerable variation in thyroglobulin concentration in blood of the thyroid vein from time to time, and on the two sides under presumably uniform experimental conditions. We do not know, at present, whether this is due to variations in the blood flow or to actual variations in rate of thyroglobulin output. But it is obvious that experimental interference with the thyroid gland must yield increase in thyroglobulin concentrations greater and more consistent than the above spontaneous fluctuations, if the variations are to be given positive interpretations.

EXPERIMENTAL RESULTS. The results on nine dogs are presented in table 1. In every case tests were made on both lobes of the thyroid gland. When more than one experiment was tried on any one dog 30 to 45 minute intervals were allowed between each experiment. The + sign means an increase in thyroglobulin as compared to previous sample, 0 means no change, and − indicates a decrease in thyroglobulin titre. Two points seem significant in these results, namely, the variability in the thyroglobulin titre, and the fact that none of the variations following the specific experimental interferences exceeded the spontaneous variations in the two control dogs 33 and 34. The results therefore permit no positive conclusion. We were unable to definitely increase the thyroglobulin output or concentration in these experiments.

The results in dog 35 are given separately and in detailed figures, as this is the only experiment in which the variations exceeded that of the controls. The blood samples were drawn at intervals of 30 minutes.

Dog 35 { Left thyroid vein: 8 (control). After 30 min. stim. of cervical: 16, 32, 16, 16
Right thyroid vein: 4 (control). Sympathetic nerve: 16, 16, 8, 8

This experiment seems positive enough and the results on the two thyroid lobes run fairly parallel. The increase persisted for at least two hours after ending the nerve stimulation. It is difficult to interpret the results as due to a diminished blood flow through the gland lasting for such a long

time. But one apparently positive experiment and nine negative ones leave the question open.

In one dog (no. 36) the first samples of thyroid vein blood gave no test for thyroglobulin. Sympathetic nerve stimulation (30 minutes) and adrenalin (5 cc. 1–20,000) failed to raise the thyroglobulin in the blood to the test level.

TABLE 1

Precipitin tests for increase in thyroglobulin concentration in thyroid vein blood under the given experimental conditions

DOG	STIMULATION OF CERVICAL SYMPATHETIC NERVE	ADRENALIN	PILOCARPINE	MASSAGE OF THYROIDS
27	Left 0	0		+
	Right 0	0		+
28	Left 0	+		0
	Right +	0		0
29	Left 0	0		+
	Right 0	+		−
30	Left 0	0	+	−
	Right +	+	−	−
31	Left		+	+
	Right		+	
32	Left		0	
	Right		+	
37	Left 0		0	+
	Right 0		+	0
38	Left +			0
	Right +			+
Summary	Positive = 4	Positive = 3	Positive = 5	Positive = 7
	No change = 8	No change = 5	No change = 2	No change = 5
			Decrease = 1	Decrease = 2

+ = increase in thyroglobulin as compared to previous sample; 0 = no change in thyroglobulin content; − = decrease in thyroglobulin content.

SUMMARY

In dogs under barbital anesthesia and with the cervical sympathetic nerves severed, there are considerable spontaneous variations in the concentration of thyroglobulin in the blood of the thyroid veins as determined

by the precipitin method. These variations appear not only in different dogs, but in the blood from the two thyroid lobes of the same dog, and in the blood from the same lobe during the course of a single experiment. In experiments on nine dogs the sympathetic nerve stimulation, direct thyroid massage and intravenous adrenalin and pilocarpine failed to increase the thyroglobulin in the blood of the thyroid vein above that of the variations in the controls. In one experiment stimulation of the nerves to the thyroid was followed by an increase in the thyroglobulin concentration greater than in the controls. This may be due either to increased rate of thyroid secretion or to decreased blood flow through the gland.

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VASOMOTOR FIBERS IN RETINAL, CHOROIDAL AND CILIARY ARTERIES

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In a previous article (1) we have shown that changes in intra-ocular pressure are due solely to changes in systemic blood pressure. With this in view we have undertaken a series of experiments to study the changes in the blood vessels of the globe. Our studies were made by directed observation of the choroidal and retinal arteries with the large Güldstrand (Bausch & Lomb) electric ophthalmoscope and the ciliary arteries with the (Bausch & Lomb) silt lamp. The animals used were white rabbits and dogs under light chloretone or ether anesthesia. Some of the observations were made with the pupil dilated with atropine.

OBSERVATIONS ON RETINAL VESSELS. *A. Drugs:* The retinal arteries of the rabbit's eye being examined with the Güldstrand ophthalmoscope showed, after intravenous injection of 1 cc. of 1/10,000 to 1/5,000 adrenalin hydrochloride, that a temporary constriction followed within a few seconds by a flushing. The retinal veins were dilated immediately from the beginning.

With 2 mgm. of nitroglycerine injected into ear vein of an albino rabbit, the retinal arteries dilated slightly with very little change in the veins.

With ½ cc. of 1 per cent nicotine intravenously, the retinal arteries showed very marked blanching while the veins became flushed.

Pituitrin and ergot caused practically no change in retinal vessels.

In the dog the same changes in retinal arteries and veins were found as described for the rabbit.

B. Nerve stimulation. 1. Rabbit. With the animals under light chloretone anesthesia, the sympathetic and vagus nerves were isolated and divided.

Stimulation of the central sympathetic nerve, with a weak tetanizing current, produced a marked constriction of the retinal arteries with no marked change in the veins.

Stimulation of peripheral sympathetic caused no change.

Stimulation of the central end of vagus also produced no change.

Stimulation of the peripheral end of vagus caused a blanching of not

only retinal arteries but of the entire fundus, which did not occur after atropine.

2. Dogs. With dogs under light ether anesthesia and with artificial respiration, the vago-sympathetic nerve was traced to the first cervical ganglion where the true sympathetic nerve was isolated and divided.

Stimulation of the central end of the pure sympathetic nerve gave a marked constriction of the retinal arteries. Stimulation of the central vagus produced no change. These and all other changes were the same as in the rabbit.

OBSERVATIONS ON THE CHOROIDAL VESSELS. As the choroidal vessels of the dog could not be seen with the Güldstrand because of the deep pigmentation of retina all the observations of choroidal vessels were made on albino rabbits.

TABLE 1

Retinal vessels (dog and rabbit)

DRUG OR NERVE STIMULATION	DOSE	CHANGES IN	
		Arteries	Veins
Adrenalin (1:5000)	1 cc.	Constriction	Dilatation
Nitroglycerin	2 mgm.	Dilatation	None
Nicotine	5 mgm.	Constriction	Dilatation
Pituitrin	1 ampule	None	None
Ergot	1 ampule	None	None
Stimulation central sympathetic nerve		Constriction	None
Peripheral sympathetic		None	None
Central vagus		None	None
Peripheral vagus		Blanching	Blanching
Peripheral vagus after atropine		None	None

A. Drugs: Injections of 1 cc. of 1/5000 to 1/10,000 adrenalin hydrochloride intravenously produced a marked constriction of the choroidal arteries, followed in a few seconds by a dilatation. The veins were dilated immediately after injection. The flushing of the entire choroidal system was so marked that at first we were led to believe that the arteries dilated after adrenalin, but on closer study, with 40 times magnification, we differentiated the small choroidal arteries from veins and found that these vessels first constricted and then became dilated, while the veins were markedly dilated from the beginning.

With 2 mgm. of nitroglycerine the arteries became dilated while the veins were slightly blanched.

With $\frac{1}{2}$ cc. of 1 per cent nicotine the choroidal arteries constricted while the veins showed a very marked flushing.

Pituitrin and ergot produced no change.

B. Nerve stimulation: With the method mentioned above the central sympathetic stimulation produced a constriction of the arteries with no change in the veins.

With peripheral vagus stimulation there was a marked blanching of all vessels of the fundus which did not occur after atropine.

Stimulation of both central vagus and peripheral sympathetic produced no change in choroidal arteries.

CILIARY VESSELS. All observations of the ciliary vessels were likewise made on the albino rabbit under light chloretone anesthesia using the slit lamp.

Injection of 1 cc. of 1/10,000 adrenalin hydrochloride into ear vein produced a marked constriction of the ciliary arteries.

Inhalation of 1 pearl of amyl nitrate, or 2 mgm. of nitro-glycerine intravenously gave a marked dilatation of the ciliary arteries.

Stimulation of the central sympathetic produced a marked constriction of the ciliary arteries.

Stimulation of the central vagus produced no change.

Stimulation of peripheral vagus gave slight paleness followed by flushing which did not occur after atropine.

Stimulation of peripheral sympathetic showed no change.

MECHANICAL INTERFERENCE OF BLOOD SUPPLY. Constriction of the common carotid artery on the same side as the eye under observation produced marked blanching of the retinal and choroidal arteries which must be distinguished from a vasoconstriction. Pressure on carotid of opposite side produced a slight blanching of all arteries only.

SUMMARY

In a survey of the literature, no reference was found on the vasomotor innervation of the choroidal and ciliary blood vessels. However, histological preparations have shown the presence of nerve fibers in the cerebral vessels (2). Occasional references to the retinal innervation were simply conclusions drawn from observations on the cerebral arteries, on the assumption that both vessels acted the same because of their like embryological origin. Wiggers (3) has shown that perfusion of the cerebral vessels with adrenalin causes constriction of these arteries. Inasmuch as adrenalin acts by stimulating the myoneural junction of the true sympathetics, the conclusion is drawn from Wiggers' experiment that the cerebral vessels have vasoconstrictor fibers. Stewart (4) states "the retina, which from the standpoint of development is a portion of the brain, is undoubtedly supplied with vaso-motor fibers which run in the cervical sympathetic." Biedl and Reimer (5) found that "the cerebral vessels may be induced to contract on the application of adrenalin."

As early as 1868, Hippel and Grünhagen (6) showed that stimulation of the sympathetic nerve produced a constriction of the ocular vessels. Their evidence was drawn indirectly from observing that occasionally sympathetic stimulation caused a decrease in intra-ocular pressure, from which they concluded that the ocular vessels must have decreased in caliber.

Doyon (7), however, showed that stimulation of the cephalic end of the vago-sympathetic nerve in dogs produced dilatation of the vessels of the fundus, which was examined by a hand ophthalmoscope. Hence he concluded that there were vasodilator fibers in the vago-sympathetic nerve to the eye. A few years later, Angelucci (8) demonstrated that extirpation of the superior cervical ganglion of dogs produced a dilatation of the vessels of the iris. Thus he indirectly showed the loss of tone in these vessels after cutting the nerve, and assumed that there were vasoconstrictor fibers present.

Later, Magitot (9) stated that the uveal tract is only an erectile tissue, a true blood reservoir, in which the more or less great distention of the vessels governs the intra-ocular pressure. These vessels are influenced by vasomotor nerves. However, he gives no definite evidence for such a statement.

Hirschfelder (10) compared the action of a series of drugs (adrenalin-nitrites, etc.) on the arteries of the pia and retina and found that adrenalin constricted and the nitrites dilate both sets of arteries. Lastly, Adler (11) showed by an elaborate experiment that the rate of formation of intra-ocular fluid is diminished during stimulation of the cephalic end of the sympathetic nerve, from which he draws the conclusion that there are vasoconstrictor fibers in this nerve to the vessels of the eye. In another paper he showed that the caliber of the intra-ocular vessels is controlled by these vasoconstrictor fibers in the sympathetic nerve, but in either case the evidence is indirect, as the vessels in the fundus had not been directly observed.

Our results show conclusively that the retinal, choroidal and ciliary arteries have definite vasoconstrictor fibers which run in the cervical sympathetic. (We were unable to demonstrate conclusively any dilator fiber.) These have been demonstrated by the injection of adrenalin which causes a marked vasoconstriction of all three sets of arteries. What is more important and most conclusive, stimulation of the central end of the pure sympathetic nerve in both rabbits and dogs produces a marked vasoconstriction in all three sets of arteries.

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THE TETANIC NATURE OF THE KNEE-JERK RESPONSE IN MAN

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Present physiological thought holds that the knee-jerk is a reflex "in which a single nerve impulse is sent out from the cord, causing a simple contraction in the muscle affected."[1]

Snyder (1910) and Hoffman (1910) have indicated that the time intervening between the stimulus and the electrical response in the muscle is sufficient for a reflex and much too long for a direct excitation. The time interval according to these observers lies between 0.0113 and 0.024 second.

Snyder, by means of the thread galvanometer, found that the thigh muscles never produce anything more than a single diphasic deflection of the galvanometer thread. An examination of his three records will show, however, that in every instance there is a vibration of the string following the single diphasic deflection to which he refers. In the first record the second deflection occurs about 0.23 second after the initiation of the first; in the second record about 0.10 second and in the third about 0.12 second. The electrical changes responsible for the two deflections in each record occur in the case of the first record at the rate of approximately 4.4 per second; in the case of the second at the rate of 10 per second; and in the last record at about 8.2 per second. The second vibration of the thread in the last two records is thought to occur too soon after the administration of the stimulus to be "tetanus" rhythms produced by the subject in an effort to control the swinging of his leg.

In the absence of any indication on Snyder's records of the instant of the jerk this conclusion was reached from determining the average latent time of the knee-jerk response for eight of our subjects. It was approximately 0.08 second. This is about 0.03 second less than the interval between the application of the stimulus and the second deflection in the second record and 0.05 second less than the interval between the application of the stimulus and the second deflection of the third record. In order

[1] Howell, W. H. A text-book of physiology. W. B. Saunders Co. 1924.

for a subject to control voluntarily the movements of his leg he must first of all be made aware that the leg is moving. That is, the stimulus for voluntary effort at control must be the extension of the foot, which merely means a stimulation of kinesthetic receptors. Now the intervals 0.03 and 0.05 second which intervene between the instant of the jerk as arrived at by our averages and the second deflection in the second and third records are extremely short for reaction times. Especially is this true in this type of experiment where no emphasis whatsoever is placed upon speed or even control, for that matter. Further our experience leads us to believe that voluntary control is not exercised until after the foot starts back, a considerable fraction of a second after initiation of extension. It would seem, therefore, that the second deflections in Snyder's two last records belong to the knee-jerk phenomenon proper.

Also Snyder's pictures show that a single vibration of the thread occupies between 0.045 and 0.095 second. In other words the thread would vibrate between 10 and 22 times per second. There are two possible explanations of this frequency: 1. The electrical changes in the muscles were of the slow frequency as pictured in the record, or 2, the frequency recorded represented approximately the natural period of the thread and no necessarily close correspondence with the actual frequency of the electrical changes. Studies of electrical changes in muscles have shown that the oscillations vary between 50 and 500 per second so that a slow rate of 10 or 20 per second appears to be far below the range usually accepted. If this slow rate represents the natural period of the thread then higher frequencies, which all studies have demonstrated to exist, would be suppressed and the record would give only an indication and not a detailed picture of the electrical changes in contracting muscle. The discrepancy between the records relative to the length of time occupied by a single deflection may be accounted for on the basis of a difference in tension of the thread for the different experiments. A change in tension, of course, gives a change in the natural period of a vibrating element.

These considerations and the fact that a refined technique was available for making a study of electrical changes in the quadriceps femoris muscle during the knee-jerk response prompted the writers to undertake the present study.

METHOD. A five-stage amplifier was used in these experiments.[2] The first three stages were of the impedance coupled type comprising one stage of high and two of low amplification. These three stages which acted as

[2] It does not seem wise to give a detailed description of the amplifier. Such a description in order to be of value would have to be highly technical and consequently out of place in an article of this type. Mr. Hunter, who is a physicist and a radio engineer of wide experience, designed the amplifier especially for this type of work and has operated it during every experiment.

the input unit were completely shielded to eliminate magnetic and electric fields. The second or output unit comprised two stages of the transformer coupled type. A volume control was placed at the second tube of the three-stage unit in order to insure against distortion arising from overloading the tubes. An input transformer was used in all instances.

A phonelescope[3] with an electro-magnetic type of telephone receiver screwed into its back served as the recording instrument.

Time was recorded by running a sixty cycle current through a step-down transformer (110/24) and a 2 m. f. condenser to a phonelescope with an electro-magnetic type of telephone receiver. Daily frequency-meter records show that the alternating current never varies more than 0.8 of 1 per cent. This variation is negligible as far as our records are concerned.

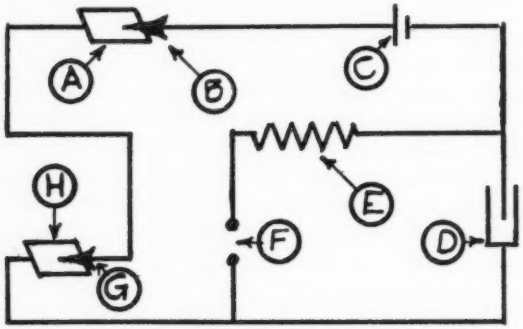

Fig. 1. A wiring diagram of the signal circuit. *A* is the thin iron plate over the patellar tendon; *B* is the magnetic hammer; *C*, the dry cell; *D*, the 4 m.f. condenser; *E*, the 200 ohm resistance; *F*, the phonelescope; *G*, the magnetic stirrup; and *H* is the heel plate.

Eastman standard size, super-speed moving picture film was used. The electrodes were two German silver plates 27 mm. in diameter covered with canton flannel which was soaked in a concentrated saline solution. One electrode was placed on the center of the rectus femoris muscle and the other at the central end.

A wiring diagram of the signal circuit is shown in figure 1. A make and break circuit operated a phonelescope with the electromagnetic type of telephone receiver to furnish the signals. When the circuit was closed by

[3] "Phonelescope" is the trade name of a standard instrument designed and sold by H. G. Dorsey, 1 Essex Avenue, Gloucester, Mass. Briefly it is an optical lever. A very small mirror mounted on jewel bearings is activated by a sensitive membrane. A beam of light focused upon the movable mirror is reflected to the photographic film.

striking the ligamentum patellae a lateral movement of the phonelescope mirror was produced. The mirror remained in the second position until the circuit was broken by extension of the foot. This permitted the mirror to return to its original position. Thus when a beam of light was focused upon the movable mirror and reflected on the moving film two movements were recorded; the first for the make or the stimulus and the second for the break or response. To aid in maintaining contact immediately after the patellar tendon had been struck, both the hammer used to elicit the response and the foot stirrup carried a small permanent magnet. The foot stirrup magnet offered no appreciable resistance to the jerk. When the circuit was closed by striking the thin iron plate placed over the center of the ligamentum patellae with the magnetic hammer there was produced a small stray electrical field which disturbed slightly the amplifier in some instances. In order to reduce this disturbance to a minimum a 200 ohm resistance was placed in series and a 4 m.f. condenser in parallel with the phonelescope. A piece of dental rubber dam was placed between the knee and the iron plate to insulate the subject.

That this type of amplifier and recording device are free from inherent periodicities is shown by figure 2 which is a picture taken with the input of the amplifier short circuited. It is seen that the apparatus was perfectly quiet.

The subjects were comfortably seated in a chair with the leg supported in a slightly elevated position so as to bring some tension on the rectus femoris muscle.

DATA. Twelve normal male subjects placed under controlled conditions supplied the data. From ten to fifteen records were taken from each individual. Periodic discharges have never been found absent in any subject. Some of the records showed only one volley but the majority gave several. The five records of action currents during the knee jerk response reproduced in this paper are typical.

Figures 3 and 4 present two records of the same subject. In figure 3 there occurred two envelopes[4] within the latent time of the extension of the foot and one after the foot got under way. These three envelopes appeared regularly at the rate of 12 or 15 per second. The envelopes are not of equal duration, the second being the shortest and the third the longest.

[4] In a previous communication (This Journal, lxxxi, 355) two of the authors (L. E. T. and T. A. H.) presented action current records which showed "a modulated wave, that is, a wave having the amplitude of its oscillations varied periodically." The modulated wave was made up of "an audible or principal frequency and an inaudible frequency." The inaudible frequency (10 or 12 per second) was composed of the periodic variations in intensity of the audible frequency (300 to 600 per second) and was termed the "envelope." It is considered that the present records show the same phenomena and that the term "envelope" should be used.

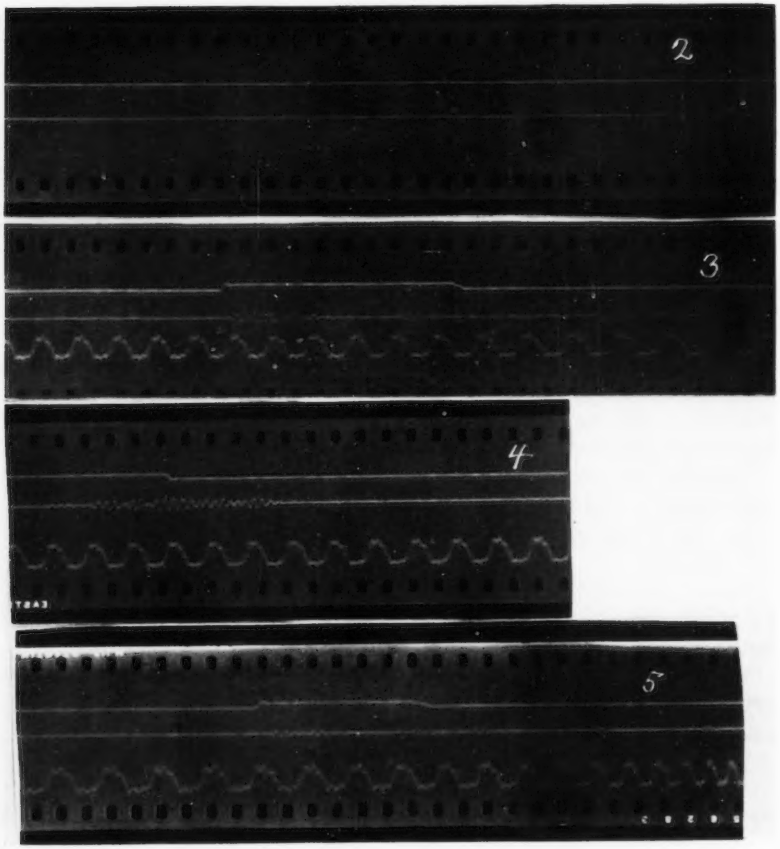

Fig. 2. The input of the amplifier short-circuited. The first line above the time line is controlled by the output. The second line is to be disregarded in this record.

Fig. 3. Action currents from the quadriceps femoris during a knee-jerk response of subject A. The instant of application of the stimulus and the moment of the jerk of the foot are shown on the signal line above. Time is indicated below in sixtieths of a second. The partials in the time line on this and subsequent records are due to the fifth harmonic of the alternating current.

Fig. 4. Action currents from the quadriceps femoris during a knee-jerk response of subject A. Only the instant of the extension of the foot is shown.

Fig. 5. Action-currents from the quadriceps femoris during a knee-jerk response of subject B.

The third shows signs of interruption at two points but is considered to constitute one grouping of oscillations. The extension of the foot occurred just after the second envelope.

Figure 4 is included to show a response wherein only one envelope of long duration appeared. It occupied about $\frac{1}{10}$ of a second and in general shows quite well the characteristic envelope boundaries. The extension of the foot occurs midway in the envelope, i.e., at the instant the oscillations reach their maximum intensity.

In figure 5 the envelopes are less periodic but well shown. There is a possibility that the second and third are really one grouping which was interrupted. If this is the case then the envelopes occur quite regularly at the rate of 15 to 20 per second. In any event the extension of the foot occurred during the second envelope.

Figure 6 shows a long envelope preceded about $\frac{1}{15}$ of a second by a brief one. The extension of the foot as in figure 4 occurs midway in the long envelope.

Three envelopes are presented in figure 7. They occur at the rate of about 15 per second. Each succeeding envelope is longer than the preceding one until the last one occupies about $\frac{1}{2}$ of a second. The extension of the foot occurs between the first and second envelopes.

All of the knee-jerk records that have been obtained indicate that there may be one or two envelopes within the latent time of extension of the foot. The number varies not only for different individuals but for the same individual at different times. Also the envelopes present a variation in duration ranging from about 0.0166 to 0.10 of a second. They appear to occur at a rather constant rate, however, for each record.

No consistent temporal relationship seems to exist between the appearance of an envelope and extension of the foot. The foot may be extended during an envelope or between envelopes.

In order to compare action currents during the knee-jerk with those during voluntary contraction figures 8 and 9 have been included. Figure 8 is a typical example of action currents occurring late in a knee-jerk record. Five envelopes are shown. Figure 9 is a picture of action currents from the quadriceps femoris during voluntary extension of the foot. Here are presented three more or less well defined envelopes occurring at a rate of about ten per second.

Discussion. Studies carried on by Piper (1912), Forbes (1924), Cobb (1918), Adrian (1925), Athanasiu (1923) and others have demonstrated that all voluntary contraction gives rise to an action-current which has for its chief characteristic the presentation of a number of oscillations varying between 50 and 500 per second. Athanasiu found in action-currents from the forearms during voluntary flexion of the digits a periodic grouping of the oscillations, the groups occurring about ten times per sec-

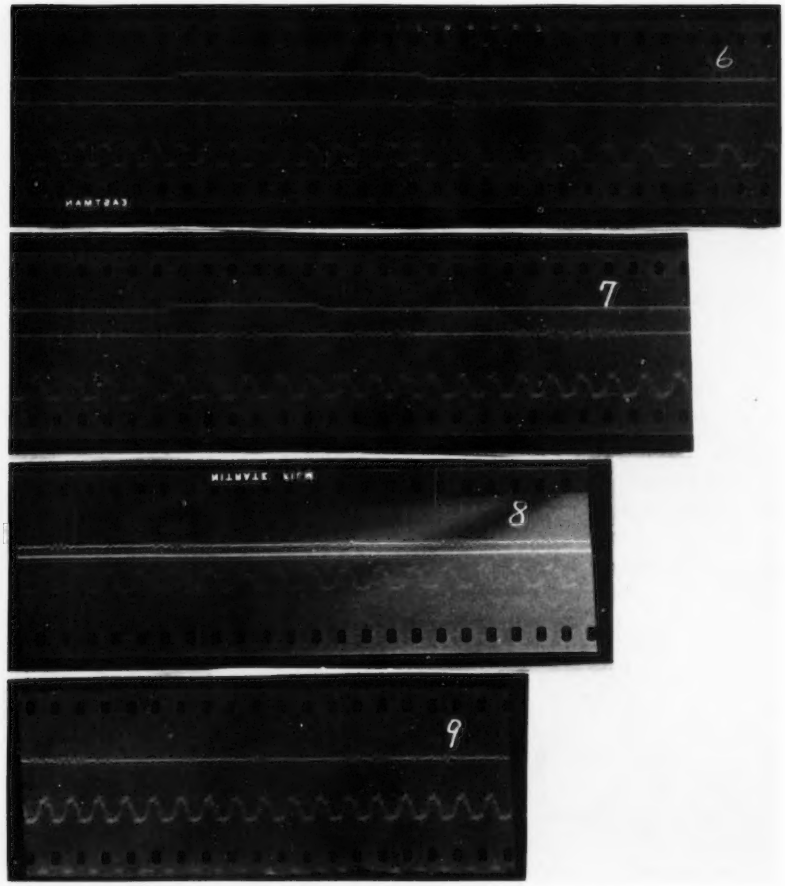

Fig. 6. Action currents from the quadriceps femoris during a knee-jerk response of subject C. The oscillations immediately below the instant of application of the stimulus are due to disturbances in the amplifier caused by the making of the signal circuit. They need not be confused with the action current envelopes which occur later in the record.

Fig. 7. Action currents from the quadriceps femoris during a knee-jerk response of subject D. This record also shows the disturbance in the amplifier due to the making of the signal circuit.

Fig. 8. Action currents from the quadriceps femoris muscle occurring late in a knee-jerk record of subject B.

Fig. 9. Action currents from the quadriceps femoris muscle during voluntary extension of the foot by subject B.

ond. Travis and Hunter (1927) confirmed Athanasiu's findings in that the action current records of voluntary contraction presented a modulated wave, having the amplitude of its oscillations varied periodically.

Also action currents from voluntary contraction of the quadriceps femoris in the extension of the foot as shown in figure 9 bear out this modulated wave characteristic.

Now the records of action currents during the knee-jerk response as presented in this study show a marked resemblance to records of action currents during voluntary contraction. Only one difference seems to appear and that is the envelopes in the former records are more distinctly separated than those in the latter records. This striking similarity between the two types of records might be explained on the basis that the action currents in both instances are due to the same thing, viz., a tetanus provided by the central nervous system. That the records of the knee-jerk are not records of voluntary contraction is indicated by several facts: 1. Over half of the records showed two envelopes within the latent time of the extension of the foot. 2. A break occurs in all of the records in the form of an absence of action-currents from a point about 0.15 second after the jerk of the foot to a point about 0.35 second after the jerk. The first point is taken to indicate cessation of action-currents in the knee-jerk while the second is assumed to indicate the beginning of the action-currents of voluntary contraction. That is, the action currents of the knee-jerk response proper are thought to continue on the average about 0.15 second after initiation of extension of foot. 3. The envelopes after they begin continue to appear at a more or less regular rate past the instant of the jerk of the foot.

These considerations quite well establish the fact that the action currents in the knee-jerk records belong to the knee-jerk phenomenon proper and not to voluntary contraction.

Pictures of action currents occurring sufficiently late in the records to place them undoubtedly in the voluntary effort group are, however, practically identical with those of the action currents of the knee-jerk response proper. A comparison of figure 8 with figures 3, 4, 6 and 7 confirms this statement. It would seem, then, that as far as the records are concerned there is no fundamental difference between the two types of electrical change in the quadriceps femoris; the one, produced by stimulation of the organs of Golgi to produce the knee-jerk and the other either by kinesthetic stimuli to produce voluntary control or by voluntary effort.

It was brought out in a previous communication[5] that the action currents of voluntary contraction are probably due to two or more discharging centers, "one furnishing the audio or principal frequency and the other

[5] Travis and Hunter. *Loc. cit.*

the modulating inaudible frequency which causes the envelopes." Inasmuch as the knee-jerk response records present the envelopes it would seem reasonable to suppose that here also are indications of at least two discharging centers.

Just where these centers are in the central nervous system is beyond the scope of this study. It merely presents material which points to the fact that there are two or more discharging centers operative in the knee-jerk response of man and that this type of response is very similar to a voluntary one in being tetanic in nature.

CONCLUSIONS

1. Action currents recorded from twelve normal male subjects showed, without exception, periodic discharges within the latent time of the knee-jerk. These indicate that this reflex is not a simple muscle twitch but a tetanic response.

2. The data show that action currents set up in case of voluntary movements are essentially the same as those in reflex responses.

3. The data suggest that there are two or more discharging centers operative in the knee-jerk and that this type of response is very similar to a voluntary one, in that it is tetanic in nature.

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BLOOD PRESSURE IN THE RAT

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During the course of a series of researches on the physiology of the rat carried on in this Department it became desirable to know whether there are any correlated changes in blood pressure. It was also felt that information on the subject would be of general interest considering the present widespread use of the rat as an experimental animal. The success achieved has been gratifying and indicates the feasibility of substituting rats for cats and dogs for use in experiments of this general nature in locations where the latter animals are expensive or difficult to obtain. Such a substitution should improve the quality of mammalian research since the rat can be grown easily under well-controlled conditions and fairly free from infection and in these respects is distinctly superior to stray animals commonly used.

The only record found of previous investigations is that published in 1924 by Baldwin and co-workers concerning the variations in blood pressure in the rat fed on various deficiency diets. These data will be considered later.

The albino rats used as experimental material were raised in the departmental colony from breeding stock obtained from the colony of the Wistar Institute. While animals of both sexes and all ages (from 4 weeks to 2 years), including some used in other experiments, have been studied, only such data will be considered in this paper as have been secured using normal adult animals. For this purpose only animals above 6 months in age were used. Hitchcock (1926) has shown that the average maximum weight and activity are attained at this age. Certainly reproduction is possible at a younger age but the litters are usually small. Lee (1926) has found that the metabolic rate does not become constant until the sixth month.

Blood pressure was measured and recorded by cannulating the abdominal aorta or the iliac or carotid artery. The last named artery was generally employed because of the greater ease and lesser traumatism in that location than is necessarily involved in entering the abdominal cavity. Cannulation of the arteries of the smaller animals was done by aid of a binocular dissecting microscope. Connection with the vessels was made by means of metal cannulas improvised from standard Luer

needles and described elsewhere (1927). The cannula commonly used in adult rats was made from a gauge 20 needle. In a few cases needles 2 sizes smaller or larger than this were used. We have found such a difference in sizes of cannulas not to affect the observed pressure.

Ether and amytal (iso-amyl barbituric acid) injected subcutaneously and intra-peritoneally were used singly and together as anesthetics. The action of these drugs upon the circulatory system is recognized and will be given due consideration in evaluating the data.

The animals were operated upon as quickly as possible after surgical anesthesia was obtained, a cannula inserted into the artery chosen and blood pressure recorded in the usual way by means of a mercury manometer. It was found possible to reduce the whole procedure, from the time ether was given until the recording was begun, to 6 minutes. The time element up to one hour was found not to be important in influencing the determination. In this manner records of thirty minutes' duration were made with ease. Feeling it desirable to secure more extended records, intravascular injections of heparin (0.1 mgm. per 100 grams body weight) were employed to delay clotting. The substance was dissolved in approximately 0.5 cc. distilled water, normal saline or even tap water and injected intravenously or through the carotid cannula. By making the injections while recording blood pressure we found no effect upon pulse rate, respiration or blood pressure, thus showing that Reed's observations upon the effect of this substance in cats and dogs can be extended to include the rat (1925). By the aid of this substance we were able to secure records of 45 minutes' duration and by removing the clot which ultimately formed in the cannula to extend the time to 2 hours. A record of greater duration than this was not attempted.

Blood pressure in normal animals. In all 41 animals, 23 females and 18 males were used. One determination was discarded because of difficulty with clotting. In every case a record of the blood pressure was made of several minutes' duration. In the majority of cases the records show a constant pressure (fig. 2). Occasionally a fluctuation of the pressure through 5 to 10 mm. of mercury was observed in which cases an average level maintained during several minutes was considered as the correct determination. Due to the rapid pulse and respiratory rate and the inertia of the mercury the pulse pressure as registered seldom exceeded 1 mm. of mercury and the respiratory wave was seldom more than 2 mm. high.

To facilitate study and publication the data are plotted in order of increasing pressure (fig. 1). The maximum pressure in this group is 150 mm. Hg, the minimum 92 mm. Hg, and the arithmetical mean 119 mm. Hg. The arithmetical mean for males is 123.5 mm. Hg; for females 115.5 mm. Hg. In both sexes the mode follows the mean. There is no

Fig. 1. * Indicates estimated age

correlation between body weight and blood pressure; however, such a comparison is confused by the age factor. We are now engaged in the study of animals of all ages and the data so far accumulated indicate that at least up to 6 months of age there is a direct correlation between blood pressure and age.

Our determinations of blood pressure do not agree with those made by Baldwin who found the normal rat to have a minimum pressure of 72 mm. Hg; a maximum of 92 mm. Hg, and an average pressure of 84 mm. Hg. Two distinct differences in procedure suggest themselves to explain this discrepancy in observations. First is the difference in age of the animals used. Baldwin employed animals of approximately 11 weeks. Using the procedure described above we find the average blood pressure in 8 rats of this age to be 100 mm. Hg. This accounts for slightly more than half of the difference between the average observations. Second is the anesthetic used. While it is recognized that ether in the third stage of anesthesia slightly lowers the blood pressure of human beings, only a slight temporary effect can be shown upon the blood pressure of the rat by changing the intensity of the anesthesia. Therefore, the adult rat's normal pressure is probably very close to 120 mm. Hg. On the other hand chloral hydrate which was used by Baldwin is recognized as a strong circulatory depressant, paralyzing the vasomotor system. To find out the extent of the effect on rat blood pressure two experiments were performed.

The anesthetic dose of chloral for rats was found by trial to be 0.03 gram per 100 grams body weight. Fifteen-hundredths (0.15) gram of chloral dissolved in 5 cc. water was injected high into the rectum of a 300 gram rat. Surgical anesthesia was produced in 35 minutes. Nine minutes later the animal's blood pressure was recorded from the carotid artery and found to be equal to 85 mm. Hg. The anesthesia at this time was very light, the animal moving occasionally. A second mature rat weighing 374 grams was anesthetized with ether and the blood pressure by the direct method found to be equal to 118 mm. Hg. Recording was continued and 0.06 gram chloral hydrate dissolved in 2 cc. water was injected by catheter into the stomach. Sixteen minutes later the blood pressure had fallen to 87 mm. Hg, where it remained for 18 minutes. It then rose slowly to reach the original pressure 26 minutes later. These observations indicate that chloral exhibits its characteristic mammalian effect upon rats and therefore explains the low pressure observed by the above mentioned investigators.

Seventeen rats of this series were injected subcutaneously with amytal (50 mgm. per kilo) then within 15 minutes the anesthesia was completed with ether and the operation performed. (With amytal alone one-half to one hour is required to produce complete anesthesia.) The average blood pressure of animals anesthetized thus was found not to be materially

different from that observed upon animals anesthetized with ether alone. However, the pressure gradually falls through 10 to 20 mm. in long continued observations. This point is mentioned because it is known that intraperitoneal and intravascular injections of amytal produce a temporary fall in blood pressure in dogs due to its depressant action on the respiratory mechanism.

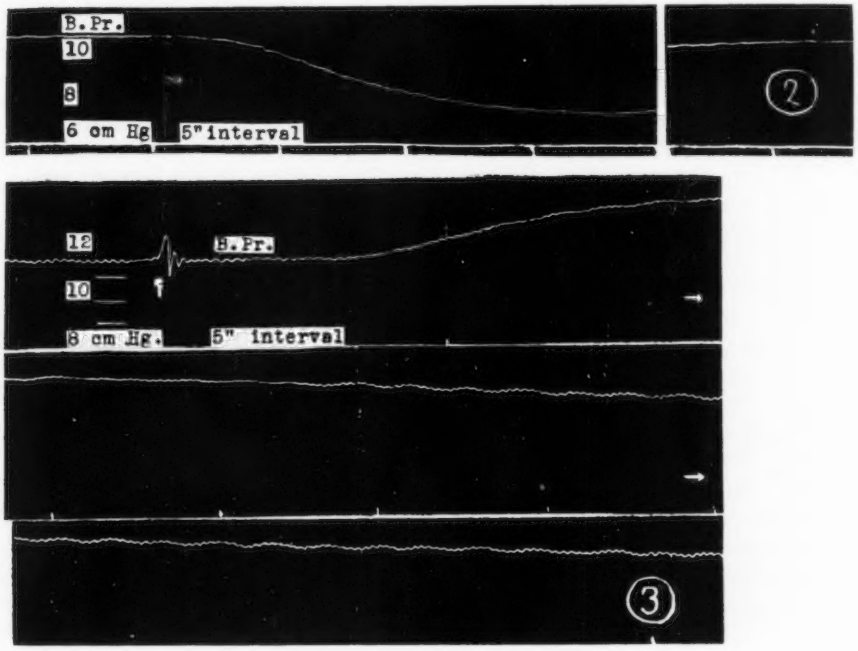

Fig. 2. Record of blood pressure of rat 11, female, weight 245 grams under ether anesthesia. The pressure was recorded from the aorta using a gauge 20 cannula. The upper trace shows pulse waves superimposed upon slower respiratory waves. The break in the record represented 45 seconds during which time the blood pressure gradually rose through the difference of levels shown.

Fig. 3. Male rat, weight 195 grams, 16 weeks old. Ether anesthesia, blood pressure by aorta. Arrow indicates injection of 0.00025 mgm. adrenalin chloride.

FACTORS INFLUENCING BLOOD PRESSURE. The effect on blood pressure of sectioning and faradizing the intact and cut vagus was observed on 13 animals of varying ages. The results obtained were consistent. In every case tying or cutting one or both vagi resulted in slowing the heart and a fall in blood pressure. Reed (1926) has shown that a rise in pressure as a result of releasing the heart by cutting the vagi in the dog occurs only if the

operating time is within five minutes, and that a small per cent of the animals always show a slowing of the heart following such procedure. No animals of this group were operated upon within the time limit prescribed by this observer. This fact probably explains our inability to show a tonic vagus effect in the rat. Faradization of the peripheral end of either cut vagus causes slowing or complete inhibition of the heart according to the strength of the stimulus. Faradization of the central end of the cut vagus, the opposite vagus being intact produces after a variable latent period the usual fall in blood pressure with slowing of the heart and respiratory rates.

That the rat has a typical mammalian vasomotor system is shown by the blood pressure response to amyl nitrite inhalation and adrenalin injection. Figure 2 shows the effect of causing the animal to breathe amyl nitrite fumes. Without a perceptible change in heart rate the blood pressure fell from 106 mm. Hg to 74 mm. Hg. The respiration rose from 48 per minute to 66 per minute. The pulse remained constant at 360 per minute. Figure 3 shows the effect of injecting intravenously 0.00025 mgm. of adrenalin (0.00050 mgm. per kilo) into the jugular vein. The heart and respiratory rates increased slightly after the injection was made. The blood pressure rose from 116 mm. Hg to 140 mm. Hg.

The adequacy of the mercury manometer for studies of this nature can be questioned. While the pulse is visible in these records satisfactory examination requires the use of a magnifying glass. We use the binocular mentioned above. The pulse rate in the rat is so high and the inertia of the recording system so great that the method described here cannot be used for the study of heart action. Pulse pressure in the rat must necessarily be small; however, the use of a recording device such as the Franck capsule should make the study of heart action no more difficult than is experienced in larger animals.

SUMMARY

1. Using the standard manometric method the arithmetical mean blood pressure in 40 adult rats anesthetized with ether was found to be equal to 119 mm. Hg.

2. The evidence indicates that this figure is fairly close to the rat's normal blood pressure.

3. The mean pressure for males is slightly higher than that for females which may be correlated with the average differences in weight of the two sexes.

4. Incomplete evidence indicates that between the ages of 4 weeks and 6 months there is a close correlation between age and blood pressure.

5. By observing the action of drugs and of electrical stimulation of the vagi the rat is shown to possess the typical mammalian vasomotor and cardio-inhibitory mechanisms.

I am indebted to Prof. R. G. Hoskins and Dr. F. A. Hitchcock for advice given during the preparation of this paper and to the latter for a number of the experimental animals used in securing the data.

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FURTHER OBSERVATIONS ON THE EFFECT OF CARBON ARC RADIATION ON METABOLISM IN THE DOG[1]

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In former publications, Mayerson, Gunther and Laurens (1925) (1926) pointed out that carbon arc irradiation of normal dogs increases endogenous metabolism and stimulates Ca and P absorption. With dosages of moderate intensities (eight consecutive exposures, each with an energy value of 55.44 gm. cal. per sq. cm. of surface exposed) serum Ca decreases while serum P rises; with excessive irradiation (110.88 gm. cal. per sq. cm. daily for 8 days) both serum Ca and P increase. Two additional observations are now submitted.

In the last series of experiments reported (Mayerson, Gunther and Laurens, 1926, experiments 3 and 4, p. 432) a short-haired, white female dog, P, was exposed to the carbon arc for 2 hours daily (energy value 110.88 gm. cal. per sq. cm.) for 8 days, followed in about 2 weeks by a similar irradiation period, with resultant loss of appetite and marked disturbance of nitrogen equilibrium. These profound effects on the general nutrition of the animal suggested that the physiological limit of dosage of this particular animal had been exceeded. Assuming that a pigmented animal might be less sensitive to the same dosage and that these disturbances might therefore be avoided, the experiment was repeated, using a short-haired brindle bull, dog A, with a short-haired brown terrier, dog B, as a control.

The Majestic Arc, or Solarlite, burning white flame carbons, was, as formerly, the source of radiation. Due to errors in calculation the values previously given for the spectral distribution of the energy are incorrect and should be as follows: 21.9 per cent is in the ultra violet; 43.5 per cent is in the visible, and 34.6 per cent is in the infra red. These are the averages of measurements made by a screen method and by a dispersion method using quartz and rock salt illuminators. The average total radiation intensity at 40 cm. is 0.912 gm. cal. per sq. cm. per minute, which is very

[1] The cost of the spectroradiometric apparatus used in these investigations was partially defrayed by a grant from the Elizabeth Thompson Science Fund to Dr. Henry Laurens.

close to Coblentz's (1920) value of 1.2 gm. cal. per sq. cm. per minute for the average normal solar radiation intensity for 3 hours at noonday in Washington, D. C. However, since not more than 1 per cent of this solar radiation is in the ultra violet, the animals were receiving about 20 times as much ultra violet per sq. cm. per minute as they would have been if exposed to solar radiation of the same total radiation intensity.

Experiment 5. The experimental procedure was similar to that obtaining in former experiments. The dogs were placed on a standard maintenance diet (Cowgill, 1923) and the N balance and the levels of serum Ca and P determined. Beginning on October 9 the back of dog A (eyes shielded) was irradiated for 2 hours (110.88 gm. cal. per sq. cm.) on 8 consecutive days. On November 9, 17 days after the last exposure, the dog was again subjected to a similar irradiation period and the analyses continued for about a week after the irradiation was discontinued. Blood samples were usually taken shortly before each exposure, in some cases they were drawn both before and immediately after.

The findings are similar to those of experiments 3 and 4 previously reported. There is evidence of slight N retention during the first irradiation period, an increased excretion in the post-irradiation period, and a markedly increased excretion and a consequent negative balance during the second irradiation period, the N excretion on the last two days of irradiation being almost double the normal values (fig. 1). The food intake throughout is constant, there being no loss of appetite or change in weight. Serum Ca and P, in general, follow the same course previously observed, except that serum Ca rises during irradiation rather than in the post-irradiation period (fig. 2). Serum P, as before, increases considerably in the post-irradiation period. When samples are drawn both before and after irradiation, serum Ca (with one exception) as well as serum P, shows lower values after than before the exposure, a decrease which has usually disappeared by the next morning. This phenomenon will be referred to below.

Experiment 6. In the experiments so far reported the back of the animal was irradiated. Dodds and Webster (1924), Cameron and MacMillan (1924) and Ivy, McCarthy and Orndoff (1924) showed that abdominal irradiation of humans and dogs with x-rays produces effects which are not obtained when other parts of the body are exposed. It seemed interesting to see if there was a similar differential action here. Accordingly, the abdomens of dogs A and B were irradiated for 1 hour daily (55.44 gm. cal. per sq. cm.) for 8 days. The results show interesting differences from those obtained on exposure of the back. While the N metabolism follows the same course, i.e., increased excretion resulting in a decreased balance, both serum Ca and P now drop during the irradiation period (fig. 3). The decrease of serum Ca in dog B is much greater than has formerly been

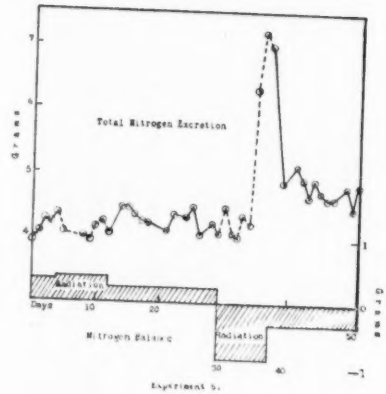

Fig. 1

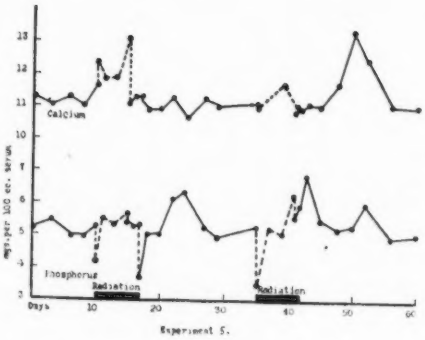

Fig. 2

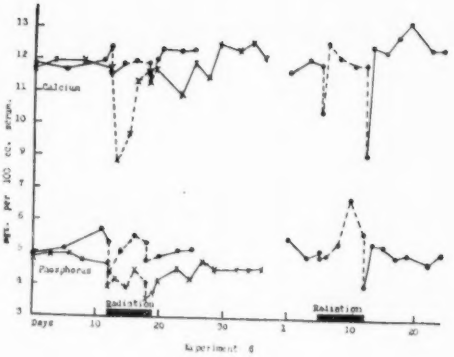

Fig. 3

Fig. 1. The effects of carbon arc radiation on nitrogen metabolism. The broken line denotes period of irradiation. Back of the animal was exposed. The dosage for each irradiation period was 2 hours at 40 cm. from the lamp (110.88 gm. cal. per sq. cm.) daily for 8 days.

Fig. 2. The effects of carbon arc radiation on serum Ca and P. The broken line denotes period of irradiation. Back of animal was exposed. The dosage for each irradiation period was 2 hours at 40 cm. (110.88 gm. ca. 1 per sq. cm.) daily for 8 days.

Fig. 3. The effects of abdominal irradiation on serum Ca and P. The values for dog A are indicated by dots, for dog B by crosses. The broken line denotes period of irradiation. The dosage for both dogs was 1 hour at 40 cm. (55.44 gm. cal. per sq. cm.) daily for 8 days.

observed. There is also a considerable drop in serum P in dog B which is only slightly recovered from, the level remaining low even 18 days after the last irradiation. In none of the previous experiments was there ever the slightest evidence of a decrease in serum P, irradiation being followed by a marked rise either immediately or during the post-irradiation period.

Notwithstanding these variations in results, a second abdominal irradiation of dog A with the same dose, 35 days after the previous exposure, elicited changes in serum Ca and P of the same order as those observed on intensive irradiation of the back, serum Ca rising slightly during the period of exposure and continuing to rise in the post-irradiation period, with serum P increasing during the exposure and decreasing to normal as the irradiation is stopped.

As noted above in experiment 5 (fig. 2), when blood samples are drawn before and after irradiation, the serum Ca (with one exception) and the serum P values after are lower than before it. In experiment 6 (fig. 3) the values of both constituents are decidedly smaller after irradiation than before. This is true even though in some cases the levels of samples drawn before are rising. This finding can hardly be due to variations in the constituents at different times of the day, for blood samples taken at the same time from the control dog show no such variations. Hjort, Robison and Tendick (1925) have also shown that serum Ca in the dog is relatively constant throughout the day. Since both constituents usually decrease as a result of the exposure, the conclusion is natural that there is an increase in blood volume caused by the irradiation and that this is recovered from by the following day. The unusual decrease in Ca and P observed in the first irradiation period of experiment 6 might in the same way be explained as a lasting dilution with no recovery between the successive exposures due to the extreme vasodilatation caused by abdominal irradiation. There is usually less hair and pigment on the abdomen of dogs than on any other part of the body and therefore less absorption of the shorter wave lengths; these penetrate further and are absorbed in greater amount by the blood of the abdominal region. The vasodilatation and erythema of the exposed region can be easily seen to be greater than when the back is exposed. The failure to observe the same effects in the second irradiation period in experiment 6 is probably due to a decreased sensitivity following the previous exposure.

The fact that the greatest changes are often evident in the post-irradiation period suggests the possibility that during the irradiation period they are much greater than actually observed, being obscured by the diluting effect of the increase in blood volume. Barkus and Balderrey (1924) concluded that the decrease in plasma P concentration obtained in sheep following prolonged carbon arc irradiation was due to dilution resulting from vasodilatation and diffusion of water from the tissues rather than to

increased excretion. Miles and Laurens (1926) also obtained evidence of an increase in blood volume. Experiments bearing on this problem are now in progress.

The changes in nitrogen metabolism are uniform throughout and occur regardless of whether the animal is irradiated on the back or the abdomen. In every case, with the smaller dose and with the more intensive exposure there is an ultimate increase in the total excretion and a shift to a negative balance either during the last part of the irradiation or in the post-irradiation period. During the first days of irradiation, before many exposures have been given, there is evidence of retention and increase in balance, findings similar to those reported by Yoshiue (1924). Without question the effects produced on the N metabolism are dependent on the dosage, excessive or prolonged exposure leading to a disturbance in absorption of nitrogen and probably increased protein catabolism or destruction of cell protoplasm. The amount of radiant energy which can be tolerated before destructive changes set in varies considerably. Much greater disturbances were observed in experiments 3 and 4 where dog P, a white, short-haired animal was subjected to excessive amounts of irradiation, than when dog A, dark and short-haired, was exposed to the same amount of radiation, indicating that pigment exerts a protection, at least to excessive irradiation.

SUMMARY

Additional observations on the effects of carbon arc radiation on the metabolism of a short-haired pigmented animal are reported. Dorsal exposure of the dog for 2 hours (110.88 gm. cal. per sq. cm.) for 8 days, followed in 17 days by a second series of similar exposures, results in an increased N excretion and a rise in both serum Ca and P, effects similar but more marked than those observed in previous experiments with moderate exposures of 1 hour (55.44 gm. cal. per sq. cm.) for comparable periods. The loss of appetite and general malaise previously obtained on irradiation of a short-haired, white dog under the same conditions are absent.

Abdominal exposure for 1 hour for 8 days produces an increased N excretion comparable to that obtained with dorsal irradiation, but there is a decrease in both the serum Ca and P in contrast to the rise in serum P obtained with dorsal exposure. A subsequent similar irradiation period gives results comparable to those of dorsal irradiation under the same circumstances.

When blood samples are drawn before and after the individual exposures, the latter sample usually shows lower Ca and P values, though the levels of samples drawn before may be rising. It is suggested that these results as well as those obtained on the first abdominal exposures are due to blood dilution resulting from vasodilatation and diffusion of water from the tissues. This effect is not as marked on dorsal exposure or in the second

abdominal irradiation period due to a lessened sensitivity of the exposed area to the radiation.

The writer wishes to express his gratitude and thanks to Dr. Henry Laurens for his help and criticism.

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THE DISTRIBUTION OF NITROGEN IN THE BLOOD AND URINE OF THE TURTLE (CHRYSEMYS PINTA)

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The comparative biochemistry of the lower vertebrates has received little attention, a fact which seems the more remarkable in view of the introduction of the modern methods of micro-analysis, which require relatively small amounts of material and thus make possible work with the body fluids and excreta of the smaller forms. Particularly is this true of the reptiles, although the other class of the Sauropsida, the birds, has been frequently studied. It was early recognized that the nitrogenous constituents of the excreta of certain lizards (1) and of snakes (2), (3) consisted largely of uric acid or its salts (ammonium urate). Weese (4) in the laboratory of the senior author (L.) was able to demonstrate that uric acid was practically the sole nitrogenous constituent of the urine of a reptile of the arid regions, the horned lizard (Phrynosoma cornutum) of southwestern United States. Snakes and lizards would seem, therefore, to resemble the birds as far as concerns the chief end product of their protein metabolism.

The scanty evidence available indicates that uric acid is of less importance quantitatively in the urine of aquatic or semi-aquatic reptiles than is generally assumed, its place as the chief nitrogenous catabolite being taken by urea. Thus Lewis (5) observed in a limited series of analyses that the amount of urea plus ammonia nitrogen in turtle urine exceeded that of uric acid nitrogen, which constituted only 19.3 and 14.0 per cent of the total nitrogen. The urea content of turtle blood was found to resemble closely that of mammals (6). Hopping (7), on the other hand, in her study of the alligator, found that urea was present in small amounts only in the blood and urine, and that the main nitrogenous constituent of the urine was ammonia, which exceeded urea in amount and the nitrogen of which comprised from 66 to 81 per cent of the total nitrogen. In the one analysis of alligator urine reported by Lewis (5), although the ammonia nitrogen was high, it did not exceed the uric acid nitrogen in amount, and was only slightly greater than the urea nitrogen. Urea was absent (7) from the blood of the alligator and the uric acid nitrogen was found to account for only about 4 per cent of the total non-protein nitrogen.

In connection with a series of studies of the comparative biochemistry of vertebrates, we have recently analyzed blood, urine, and tissues of one of the more common species of turtles (Chrysemys pinta). The studies have confirmed our former results (5) and have demonstrated that uric acid, although quantitatively more important as a nitrogenous catabolite in the organism of the turtle than in the mammalian organism, is not the chief end product of nitrogenous metabolism in this species of reptile. We have also shown the presence of urea in significant amounts in blood and urine but have not observed so marked an excretion of ammonia as has been reported for the alligator (7).

The analyses were made during the winter months, a period during which, under normal conditions, the turtle hibernates. The animals, received from a supply house, had been kept in the usual laboratory tank for varying periods of time. The animals were decapitated and the blood collected in oxalate as an anticoagulant according to the usual method. The carapace was then sawed through, the lower half was lifted, and the bladder, after ligation to prevent loss of the urine, was removed. The tissues were removed, ground with sand, and filtrates were prepared for the determination of uric acid as described by Folin and his co-workers (8). The filtrates from liver and muscle were frequently so rich in glycogen content that the determination of uric acid presented difficulties. The glycogen was therefore precipitated in 50 cc. centrifuge tubes with 4 volumes of alcohol, the precipitate was removed by centrifugation and the supernatant liquid was decanted. The decanted liquid was evaporated nearly to dryness on a water bath, the volume made up to 5 cc. and the uric acid analysis carried out by the silver urate precipitation method of Folin (9). Uric acid added to the filtrates, prior to the removal of glycogen, could be recovered almost quantitatively (95 to 98 per cent).

The blood analyses were carried out according to the usual methods of Folin and Wu, the precipitation method (9) being used for the determination of uric acid. The standard methods for the determination of the urinary constituents were employed. In every case a precipitate, identified as uric acid or its salts, was present in the bladder. In order to dissolve this precipitate and to insure satisfactory aliquots, the bladder was washed with a buffer mixture of phosphates, similar to that used in the preparation of the uric acid standard for colorimetric determination. The precipitates dissolved readily and a clear fluid was obtained.

The results of the analyses of the blood of 8 animals are presented in table 1. Except in 2 animals (2 and 3), the distribution of nitrogenous constituents does not differ materially from that of the usual types of mammalian blood. In these 2 cases, the urea nitrogen was low, both in absolute amount and in per cent of the total nitrogen. In the other animals, there is a slightly lower percentage of the total nitrogen present as urea than in most mammals. This is due to the fact that the non-

TABLE 1

The distribution of nitrogen in the blood of the turtle

NUMBER OF ANIMAL	NON-PROTEIN NITROGEN	UREA NITROGEN		AMINO NITROGEN		URIC ACID NITROGEN		UNDETERMINED NITROGEN
	mgm. per 100 cc.	*mgm. per 100 cc.*	*per cent*	*mgm. per 100 cc.*	*per cent*	*mgm. per 100 cc.*	*per cent*	*per cent*
1						1.3		
2	34.4	4.7	13.6			0.2	0.5	85.9
3	48.8	8.3	17.0	15.9	32.8	0.5	0.9	49.3
4	67.2	16.8	25.1	15.7	23.4	0.1	0.2	51.3
5	64.5	26.0	40.3	11.1	17.2	0.6	0.9	41.0
6	70.0	25.9	37.0	11.6	16.5	0.6	0.9	37.7
7	66.6	21.6	32.4	11.7	17.7	1.4	2.1	47.8
8	81.9	17.5	21.4	13.8	16.8	1.1	1.4	60.4

TABLE 2

The uric acid content of tissues of the turtle

Results are expressed as milligrams of uric acid per 100 grams of tissue

NUMBER OF ANIMAL	LIVER	MUSCLE	HEART	KIDNEY	BLOOD
1	2.17	1.04	1.79	11.73	3.79
2	2.83	0.65	0.95	4.23	0.52
3	0.45	0.52	1.85	5.35	1.44
4	0.95	0.32	1.59	3.34	0.39

TABLE 3

The distribution of nitrogen in the urine of the turtle

NUMBER OF ANIMAL	TOTAL NITROGEN	UREA NITROGEN		AMMONIA NITROGEN		URIC ACID NITROGEN		AMINO NITROGEN		CREATINE NITROGEN		UNDETERMINED NITROGEN
	mgm. per 100 cc.	*mgm. per 100 cc.*	*per cent*	*mgm. per 100 cc.*	*per cent*	*mgm. per 100 cc.*	*per cent*	*mgm. per 100 cc.*	*per cent*	*mgm. per 100 cc.*	*per cent*	*per cent*
1	284					66.3	22.5					
2	302	144.7	47.8	65.6	21.7	22.3	7.4					23.1
3	616	254.0	41.2	84.1	13.7	153.0	24.8					20.3
4	407	183.7	45.1	40.0	9.8	112.8	27.7					17.4
5–8 (composite)	292	110.5	37.8	58.6	20.5	49.3	16.9	24.3	8.2			16.6
9–21 (composite)	135.5	32.7	24.1	15.0	11.1	18.8	13.9	5.2	3.8	7.4	5.5	40.6

protein nitrogen of the blood is somewhat higher than in the blood of the usual types of mammals reported, while the urea nitrogen in absolute amount is approximately the same. The uric acid content is low and is no greater than that of man. The undetermined nitrogen is high but some

portion of this is undoubtedly due to the presence of creatine which was not determined.

The analyses of the tissues (table 2) demonstrate that the uric acid content of the kidney is notably greater than that of the other tissues or blood. This is to be expected if, as shown by the urine analyses, the kidney is secreting a urine high in its content of uric acid. Similarly the kidney tissue of mammals, in whose urine urea is the chief nitrogenous catabolite, has been observed to be higher than the other tissues in its urea content (6), (10).

In table 3 are presented analyses of the urine of 4 animals, of a composite urine from 4 animals and of a second composite urine from 12 animals. With these larger samples it was possible to make more complete analyses of the urine. Of the individual constituents, urea was present in largest amounts, although it comprised less than half the total nitrogen; uric acid was also important quantitatively, although in all cases present in smaller amounts than urea; the ammonia content was also high, due presumably to the utilization of ammonia for neutralization of uric acid. The qualitative tests for creatinine were very faintly positive, but not enough of the material giving the tests was present to permit of quantitative determination by the picric acid method. No allantoin could be found by the method of Christman (11). In this connection the absence of allantoin from the urine of the lizard (12) may be noted.

SUMMARY

Analyses of the blood, urine, and tissues of the turtle (Chrysemys pinta) are reported. It is evident that as far as concerns the nitrogenous metabolism, these reptiles are intermediate between the reptiles of the arid regions (snakes, lizards) and the mammals, since they excrete considerable amounts of both urea and uric acid in the urine.

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STUDIES IN GASTRIC DIGESTION

The Relation of Volume, Hydrogen Ion Concentration and Buffer Capacity of the Test Meal to Gastric Contents

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That HCl is secreted in the gastric juice has been known for a hundred years. How much gastric juice is secreted? What is its relation to the character of the food intake? What is the relation of acidity to the passage of food from the stomach?

The problem of gastric motility was greatly advanced by the researches of Pavlov (1) and Cannon (2). The former showed that gastric secretion varies with the type of food eaten. The latter showed by x-ray that motility is dependent upon the character of the food—fat leaving most slowly and carbohydrates most rapidly. Acidity and motility are related also: acid foods leave the stomach most rapidly. This led Cannon to his theory of the acid reflex opening of the pylorus and closing due to acid in the duodenum. Carlson (3) states that motility plays a greater part than acidity in gastric digestion.

Theoretical. To understand the process of gastric digestion it is necessary to know how much saliva was swallowed and how much fluid was secreted or absorbed in the stomach. The knowledge of the composition of the gastric juice in response to different meals and to the same meal at different stages of digestion, and the character of the chyme issuing from the pylorus are other necessary factors. Even assuming a gastric juice of constant character secreted at a uniform rate, and an absence of saliva, unless food of constant composition should leave the stomach at a uniform rate, calculation of the amount of food digested would be difficult. Such an analysis was attempted by Arrhenius (4) but, because of lack of exact quantitative information and the probable variability of all factors involved, it seems unwarranted in the present state of knowledge. What causes the variations in the type or amount of gastric secretion, what alters gastric motility and tonus and how these are related to passage of food, are questions that must first be solved.

Newer methods of gastric analysis demand a reinvestigation of the re-

lation of gastric motility and acidity. At the time Cannon made his studies, neither was there a method for measuring the pH or true acidity of gastric contents available, nor was there an adequate understanding of the nature of buffer solutions. A review of the methods for gastric analysis was given in 1920 and a method proposed for measuring pH and buffer value (6).

Definition of terms. "Buffer capacity" is used in the same sense as "buffer value" in the previous paper (6). Since that time Van Slyke (7) has defined the term "buffer value." It is measured by the amount of base necessary to cause a change of one pH. This value changes at different acidities and is maximal at the dissociation constant of the acid. In order to avoid confusion the term "buffer capacity" has been here used. This is measured by the amount of acid or alkali, or both, necessary to change the pH between given limits. The range of most buffers of physiological interest lies between pH 3.0 and 10. which represents 0.001 N HCl and 0.001 N NaOH.

If gastric contents were a definite substance of known composition as a phosphate solution, buffer capacity would be an exact measure of its concentration. However, because the proteins and other substances exhibit different capacities as buffers at different stages of digestion, the buffer capacity varying from case to case and in the same contents at different times, the buffer capacity is not constant. We have calculated "buffer capacity" in terms of 0.1 N acid and alkali per 100 cc. of contents between pH 3.0 and 9.6. In addition to the buffer capacity the volume must also be known. Volume times buffer capacity equals "total buffer."

The numerical value of the buffer capacity corresponds closely to that of the "combined acidity" in gastric contents with free HCl or to the sum of the "total acidity" plus the "acid deficit" in those with no free HCl. The advantage of this terminology is two-fold. It defines, in one term, the same value, which was expressed by three terms. It gives a different theoretical meaning to the experimental data.

Purpose. The purpose of this study was to determine the volume, pH and buffer capacity of the residue in the stomach at definite time intervals after known meals of varying amounts and concentrations. It was hoped in this way to gain information concerning 1, the amount of gastric juice secreted in response to meals of various concentrations and volumes; 2, the effect of gastric digestion on food that passes into the pylorus, and 3, the relation between the food in the stomach and that leaving it. Study of buffer value of gastric contents in conjunction with measurement of the pH and volume should offer a new method of studying gastric digestion.

PROCEDURE. Two dogs with gastric fistulae were the subjects of experiment. A complete series of observations was thus possible instead of less

intensive studies upon many animals. The dogs were females weighing about 8 and 10 kilos. The operations were performed several months prior to the date of this work. The dogs had been used for experiments on stomach contractions and had become accustomed to laboratory life.

The dogs were fed a test meal consisting of Dryco, a dried milk preparation, and distilled water. The composition of this milk powder is: fat, 12 per cent; lactose, 46; protein, 32; salts, 7; and moisture, 3. This type of meal was selected because its composition remained constant and its concentration might be varied at will. In the proportion of 8 parts of Dryco to 100 parts of distilled water, its composition corresponds to that of partly skimmed milk. The four meals used represent roughly 100 cc. and 200 cc. of both half and whole skim milk.

The pH of the test meals was determined by the method of Kramer and Greene (5). The milk was placed in collodion sacs and dialyzed The pH of the dialysate was determined by the colorimetric method.

The buffer capacity and total buffer of the meals were determined. To 25 cc. samples of a half milk meal were added a known amount of NaOH 0.1N or HCl 0.1N. The color of the dialysate was then compared with a known pH standard both containing thymol blue as an indicator. The buffer capacity was found to be 52 cc 0.1N per 100 cc. half milk (4/100) or 104 cc. 0.1N per 100 cc. whole milk (8/100).

At approximately the same time each morning the meals were given to the animals which had eaten nothing since six o'clock the previous evening. The animals were allowed to see and taste the meal and the rest was given by gavage. The dogs were accustomed to this procedure and lay quietly during the passage of the tube. Thus errors due to lack of psychic or appetite secretion or gastric juice were eliminated.

During the experiments the animals lay on the table, held down only by two loose straps around the body. In the interval between feeding and withdrawal of the meal, the dogs were covered with a sheet and lay as though asleep. Any disturbing factors such as excitement, discomfort, fear or rage, were avoided.

The stomach contents were withdrawn at 30, 60, 90 and 120 minutes through a soft rubber catheter inserted through the fistulous opening. The catheter was connected to a flask in which the contents were collected. Suction, not greater than 20 cm. of water, was applied to the flask. Distilled water was passed into the stomach through the catheter three times in 100 cc., 50 cc. and 50 cc. amounts. These washings were removed by the same technic as that for the contents. It was possible in this way to empty the stomach very thoroughly. The contents and washings were measured and analyzed immediately after removal or were placed in the refrigerator for not more than four hours.

It was found, on analysis, that the pH was the same in the filtered and unfiltered contents and washings. The buffer capacity of the filtered samples was lower than that of the unfiltered material. The filtrates were therefore used for determining the pH and the unfiltered samples, for the determination of the buffer capacity. The hydrogen ion concentration and buffer capacity were obtained colorimetrically by the method of Shohl and King (6). Samples of 1 cc. were titrated with 0.1N HCl and 0.1N carbonate free NaOH delivered from a microburette calibrated in 0.01 cc.

The amount of contents in each washing was calculated from the buffer capacity of the washing. The volumes given in the tables and charts are the sum of the contents removed and the contents calculated to be present in the washings. This method gives a very accurate value for the volume.

RESULTS. (*Note:* To simplify the tables and charts, the various test meals used will be indicated by abbreviations. For example, 4/100, 100 cc. means a 100 cc. test meal containing 4 grams of Dryco in 100 cc. of distilled water. The values used in the charts and tables are averages derived from two or more series of experiments.)

A. *The relation of digestion to time.* Table 1 gives the average values for all meals of the pH, buffer capacity, total buffer and volume at 30,

TABLE 1

The gastric contents in relation to time with various test meals

MEAL GIVEN	TIME	pH		VOLUME		BUFFER CAPACITY		TOTAL BUFFER	
		A*	B*	A*	B*	A*	B*	A*	B*
	minutes			*cc.*	*cc.*	*cc. 0.1 N*	*cc. 0.1 N*	*cc. 0.1 N*	*cc. 0.1 N*
4/100, 100 cc.	0	6.2		100		52		52	
	30	3.9	4.3	61	59	82	83	50	50
	60	2.7	2.7	26	35	54	58	17	20
	90							8	
4/100, 200 cc.	0	6.2		200		52		104	
	30	4.0	4.7	137	141	87	78	120	111
	60	4.0	4.2	87	88	80	67	61	58
	90	3.0	2.8	34	54	49	57	17	29
8/100, 100 cc.	0	6.2		100		104		104	
	30	4.1	4.1	63	72	119	104	76	74
	60	3.9	3.9	28	46	72	123	20	56
	90							12	
8/100, 200 cc.	0	6.2		200		104		208	
	30	4.1	4.3	137	151	131	115	179	175
	60	4.1	4.1	113	115	128	124	145	142
	90	3.9	3.9	89	84	72	70	64	75
	120							36	

* This represents the average of duplicate or triplicate determinations on dogs A and B.

60, 90 and 120 minutes for dogs A and B. These data are also plotted graphically in figure 1 for dog A because the results are practically the same for both. The table and chart represent, therefore, all the data obtained. The general deduction from these findings is that a relationship exists among all the factors. The shape of the curves is similar for all meals. By all the criteria, 4/100, 100 cc., are most rapidly digested and 8/100, 200 cc., least rapidly. This conclusion, that digestion is complete, is based not only upon the volume, but also upon the acidity and the buffer

capacity of the contents. Thus we have a new correlation of digestion time. The time required for the digestion of these test meals is shown to vary from 60 to 120 minutes.

The remaining deductions in this study are based upon these values. To obtain accurate insight into the mechanism of gastric digestion, it is necessary to consider the relations between meals of varying volume and concentration and their effect on the concentration, volume and acidity of the gastric contents.

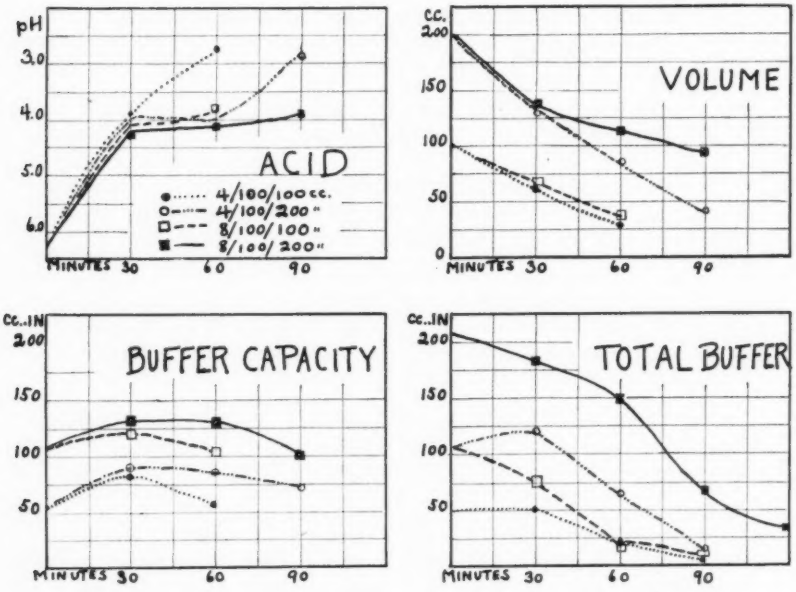

Fig. 1. Gastric contents removed after various milk test meals

B. *The effect of time on the pH of contents removed.* Figure 1 shows the average curve of the hydrogen ion concentration, expressed as pH, during the process of digestion. The pH of all the test meals at the time they were given was 6.2. During the first half-hour acidity increases in all meals to approximately the same point. The 4/100, 100 cc. meal continues to increase in acidity until it reaches pH 2.7 at the end of an hour. With the 4/100, 200 cc. meal, however, the pH remains at 4.1 for the second half-hour and drops to 2.9 at an hour and a half. The acidity of the 8/100, 100 cc. meal increases only slightly from pH 4.1 at the end of one-half hour to 3.8 at an hour. The acidity of the 8/100, 200 cc. meal does not change appreciably after the first half-hour; it decreases from 4.2

pH at a half-hour to 4.1 at an hour, and then increases slightly to 3.9 at an hour and a half.

More concentrated meals and meals of large volume become less acid in a given time, for it takes an equal amount of acid to bring either double the volume or double concentration to the same pH.

C. *The effect of time upon the volume recovered.* Figure 1 further represents the relation between digestion and volume with all meals used. As the time interval between feeding and withdrawal increases, a smaller volume is recovered. In addition, it will be seen that in the 100 cc. meals, the dilute and concentrated leave the stomach at approximately the same rate. In the 200 cc. volume, however, the dilute meal leaves the stomach at a rate much greater than that of the concentrated.

Table 2 shows percentage loss of volume for all meals. In the 100 cc. meals, the loss in volume during the first hour is practically the same for both. None of the original meal was obtained after an hour and a half. In the 200 cc. meals, during the first half-hour, the rate of loss for both

TABLE 2

The percentage loss of volume with various test meals

MEAL	30 MINUTES		60 MINUTES		90 MINUTES	
	Amount lost	Per cent lost	Amount lost	Per cent lost	Amount lost	Per cent lost
	cc.		cc.		cc.	
4/100, 100 cc.	39.1	39.1	73.6	73.6		
4/100, 200 cc.	61.4	30.7	116.4	58.2	166.7	83.3
8/100, 100 cc.	36.2	36.2	72.1	72.1		
8/100, 200 cc.	63.2	31.6	86.5	43.2	111.1	55.5

meals is about equal, but during the second half-hour the rate is three times as rapid in the dilute, and during the third half-hour, is twice as rapid in the dilute as in the concentrated.

The absolute loss in cubic centimeters for meals of the same volume during any half-hour period is greater in the 200 cc. than in the 100 cc. meals. The percentage of loss of original volume, however, during any half-hour period is greater in the 100 cc. than in the 200 cc. meals. These data indicate that with large test meals, larger volumes, but smaller percentages, leave the stomach in a given time. The 200 cc. meals take longer for digestion than 100 cc. meals, and after the first half-hour, the rate of loss is greater in the dilute mixture.

Comparing meals of the same concentration, in both the 4/100 series and 8/100 series meals, the percentage loss of the original volume is approximately the same during the first half-hour, but after that is greater in the 100 cc. than in the 200 cc. meals.

Comparing the two meals which contain the same amount of milk, 4/100, 200 cc. and 8/100, 100 cc., because of the difference in volume,

the more concentrated milk feeding is more rapidly removed from the stomach.

In meals of equal volume the more dilute meal leaves the stomach more rapidly. In meals of equal concentration, those of the lesser volume are digested more rapidly.

D. *The effect of time on the buffer capacity.* Figure 1 shows the effect of digestion on the buffer capacity of all meals. Each meal shows an increase in buffer capacity during the first half-hour. The increase is approximately the same for both 4/100 meals and also for both 8/100 meals. It remains high until digestion is practically completed.

The initial increase in buffer capacity signifies that the fluid has left the stomach and that the residue with a greater buffer capacity has remained. Following this increase, the buffer capacity (of the 100 cc.) falls more rapidly than that of the 200 cc. meals. The buffer capacity of the 4/100 tends also to decrease faster than that of the 8/100 meals.

The concentrated meal has a greater percentage loss in buffer concentration than the dilute, when test meals contain the same amount of milk.

E. *The effect of time elapsed on total buffer.* This value shows the combined effect of the test meal on the volume and concentration of the gastric residue. It measures more completely than either, the amount of material which is undigested. Figure 1 also shows the average effect of digestion on the total buffer of the contents. In the 4/100 meals the decrease of total buffer during the first half-hour is only slight; after this period the fall is more rapid, and is minimal at the end of an hour and a half.

Both 8/100 meals show a marked decrease of total buffer during the first half-hour, in contrast to the slight fall or rise in the 4/100 curves. The total buffer value of the 100 cc. meal falls rapidly to small values in an hour and a half but the 200 cc. meal is incompletely digested in two hours.

The decrease in total buffer is more rapid in the smaller meal, when the concentrations are equal.

The decrease in total buffer is the same for both 100 cc. meals at one to one and a half hours but for 200 cc. volumes is more rapid for the less concentrated meal.

F. *The relation of pH to volume recovered.* The pH tends to attain a certain level and remain there until the volume becomes quite small. At the end of digestion the acidity increases markedly, and free HCl is found.

G. *The relation of pH to buffer capacity.* The contents remain at about the same pH until a low buffer capacity is reached. At this time the hydrogen ion concentration comes to a level of pH 3.0 or less. The fall in pH occurs soonest in the contents with the least buffer capacity and the others follow in the order of their increasing buffer values.

H. *The relation of pH to total buffer.* The total buffer decreases to comparatively small values before there is an appreciable change from a fairly

constant pH to an increased acidity. The fall in pH comes about quite abruptly and occurs soonest in the meal with the least total buffer capacity. The other meals follow in the order of their increasing buffer. This indicates a direct relation between the amount of total buffer and the increase in acidity to a point where free HCl is present.

I. *The relation of buffer capacity to volume.* High buffer capacities are found in conjunction with a relatively large volume of gastric contents. Low buffer capacities are associated with small volumes. These facts are true for all the meals.

The data indicate that high buffer capacity associated with large volume is a sign of incomplete digestion. Food tends to remain in the stomach at a high buffer capacity until digestion is complete.

Discussion. Acid secretion and motility have been the center of interest in experimental gastric physiology for many years. Concentration of the meal and of the gastric contents not only gives an added insight into the mechanism of secretion and motility, but also show their correlation in gastric digestion.

Secretion. MacAdam and Bell (8) state that they obtained exactly the same acid curves on different occasions and quote references in agreement and with contrary findings. Our experience shows that the response is the same for the same dog, and is the same for both dogs.

From the buffer curve, figure 1, the amount of 0.1 N HCl in the contents can be calculated, if the pH of the meal and the pH of the contents are known. From gastric analysis alone, the secretion cannot be accurately determined; for the material digested contains an unknown amount of the secretion. Therefore, measurement of pH, like other methods of gastric analysis, gives only an indication of the amount of secretion. Davidsohn (9) and Babbott et al. (10) have reported that there is a definite increase in acidity of the contents removed, as the interval between feeding and withdrawal is increased. Our findings corroborate theirs.

Carlson (3) states that a small amount of gastric juice of relatively low acidity is constantly being secreted during fasting. The more rapid the secretion of the gastric juice, the higher is the acidity and lower its pepsin content. These facts are of interest in this study because at the time of feeding the stomach is in the fasting state.

When food is given, there is a latent period followed by an outpouring of gastric juice. The type of food has a direct bearing on the amount of gastric juice secreted. Pavlov (1) demonstrated that the most abundant secretion occurs with protein, and the least with fat. The secretion with carbohydrates falls between these. The test meals used contained very little fat and were, therefore, such as to produce maximal or nearly maximal gastric secretion.

As is well known, Pavlov has shown that secretion of gastric juice is

induced by the sight or taste of a desirable or appetizing meal. Our technic was devised so as to include the appetite secretion.

Our test meals caused a gastric secretion resulting in contents with an acidity between pH 4.1 and 3.9 in one half an hour. The amount of 0.1 N HCl secreted in the first half-hour of digestion varies between 28 and 112 cc. This fact, together with the knowledge that all the meals reach approximately the same pH at the same time, indicates a regulatory mechanism, whereby secretion is inhibited after a definite acidity is reached. It further indicates that the volume and buffer value of the meal must have a direct influence on the amount of secretion of gastric juice. Analysis of the data shows that large volumes produce more secretion than small ones, and concentrated meals stimulate more gastric juice than the same volume of dilute meals. In infants, using milk test meals, Babbott et al (10) obtained a similar increase in secretion with larger amounts of food and more concentrated food. However, the pH was not constant and the acidity fell with the increasing meals. Taken in conjunction with our work this indicates that the adult dog has the capacity to pour out the required amount of acid rapidly, whereas babies, on the other hand, have a more limited secretory capacity.

As the contents leave the stomach, materials of nearly constant composition remain until the end of digestion is approached. When a small volume and low buffer capacity are present, the acidity increases to pH 3.0 or to greater acidity. The regulatory mechanism for the stimulation and inhibition of secretion is still to be adequately described.

Motility. The volume of gastric secretion must be known in order to determine the volume of digested material. The volume of the test meal plus the amount of gastric secretion and minus the amount of material which has passed the pylorus, represents the volume of stomach contents. Gorham (11) has suggested a method for determining the factor of dilution which might be applicable in the measurement of motility. A measured amount of phenol red is added to the Ewald test meal, and the dye present in the filtered gastric contents is quantitated. This method is not satisfactory with Dryco and water test meals because dye is absorbed by the residue. Roentgen ray methods of studying motility are not quantitative and give no other factors concerned in digestion. The volume of gastric contents is the best measure of motility available at present.

The theory set forth by Cannon (2) is that the pylorus is controlled by the acidity of the contents and, therefore, influence the rate of emptying of the stomach. Marriott and Davidson (12) have shown that motility is more rapid in cases with normal acidity than in those with acid deficits. There is much similar evidence. However, this theory is giving way to a new view. Theile (13) demonstrated that alkaline test meals leave the stomach as rapidly as acid meals and hence the chemical reaction of the contents of the stomach is not the main factor in motility. Ryle (14)

believes that the relation between the tonus of the pylorus and the duodenum plays a more important part in regulating the fluid flow, than the opening and closing of the pyloric valve.

Different meals give widely divergent values. Cannon (2) for dogs, and Ryle (14) for humans, showed that proteins stimulate and fats inhibit motility, and, therefore, motility varies with the composition of the meal. Ivy (15) states that dilution of a meal with water increases the emptying time. In this work it has been shown that concentration does not affect motility in small meals. Dilute meals, however, leave the stomach at a rate much greater than that of concentrated, when large volumes are used. Thus, concentration is one factor influencing motility. When meals of equal concentration are used, the rate of emptying is greater in the small than in the large volume. Comparing the meals which contain the same amount of milk, but have different concentrations and volumes, the more concentrated milk feeding leaves the stomach more rapidly. This is of practical importance in selection of the optimal amount and dilution of food when rapid emptying time of the stomach is desired. Our experiments show that motility varies greatly with meals of different volumes and concentration.

Concentration. The Ewald test meal is a dilute test meal; test meals of other concentrations, such as the meat meal or the gruel meal, give different gastric response. Are these meals any indication of how the stomach reacts to its daily task? Is the ability to produce free HCl with tea and toast any surety of adequate gastric digestion with a hearty dinner? Unless concentration is considered, one has no insight into the gastric response with varying tasks, and hence no measure of functional capacity.

The more concentrated meals, those with a high buffer value, take longer to digest than less concentrated meals. Meals of large volume in addition to large concentration require the longest time for complete digestion. Our data on dogs are in agreement with those found by Miles (16) for babies.

Calculation of the degree of dilution from the buffer capacity of the contents might give the amount of gastric secretion. However, as in motility, the degree of dilution depends upon the rate of secretion and the rate of removal of the contents,—facts which we do not know. The estimation of the degree of dilution is further complicated because the contents are not homogeneous. This is shown by the rise in buffer value at one half hour. We interpret this rise as due to the action of the gastric juice on milk with the formation of a curd (with a higher buffer value than that of milk) which remains in the stomach, and a fluid portion which leaves it.

As digestion progresses, after the initial rise, the buffer value stays fairly constant. The middle portion of digestion involves a quantitative change in the amount and not a change in the character of the buffer substance. Large buffer capacities are associated with relatively large

volumes and indicate the beginning of digestion. Small buffer capacities in small volumes of contents signify that digestion is at an end. Therefore, the effect of concentration is important and quantitatively measurable, not only upon secretion, but upon motility.

SUMMARY

1. The amount of gastric juice secreted is sufficient to increase the pH between 4.1 and 3.9 for all meals. Therefore, the amount of HCl secreted depends directly upon the volume and concentration of the meal.

2. The pH of the stomach contents tends to remain nearly constant until the end of digestion.

3. Concentrated meals and meals of large volume take longer for digestion than small and dilute meals. The motility of the stomach, therefore, varies directly with the volume and concentration of the meal.

4. A given meal is most rapidly digested when it is concentrated in small volume.

5. The buffer capacity of the gastric contents, after an initial increase, stays fairly constant.

6. Large buffer capacities, associated with relatively large volumes of contents, indicate the beginning of digestion; small buffer capacities in small volumes of contents signify the end of digestion.

7. The effect of concentration is important and quantitatively measurable, not only upon secretion, but also upon motility.

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HICCUPS OF PHARYNGO-ESOPHAGEAL ORIGIN

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It seemed from personal experience that hiccups are often due to pharyngo-esophageal irritation. This may not account for all attacks. A survey of the literature on hiccups showed that irritation of the pharynx and esophagus as a possible cause of hiccups is frequently ignored. This work is presented because I know of no investigation on whether or not such irritation will actually cause hiccups. An investigation of after-nursing hiccups among infants is in press (1).

Rosenow (2), (3), (4), (5) concludes that the so-called epidemic hiccups are caused by a specific strain of streptococcus associated with encephalitis lethargica, apparently by an action on the central nervous system. DeBrun (6) gives a survey of causes and treatment. Knuthsen (7) gives a synopsis of over 150 cases of hiccups. Other authors are cited (8), (9), but most of the literature is merely descriptive, or deals with the therapy rather than with the causes.

Experimental. I produced hiccups in myself by drinking hot water. At first 53°C. was adequate but after several months of intermittent work 61 to 62°C. was necessary. Not more than one hiccup followed any swallow of water by this method. It was frequently necessary to swallow very rapidly many times before any hiccup occurred.

A balloon was arranged under the chin anterior to the hyoid bone and was connected with a recording water manometer. This balloon was in a position to show movements of the pharynx. The time at which the various phases of deglutition occurred was recorded by the manometer. Another balloon connected with a water manometer was swallowed and suspended at various levels in the esophagus. The tracing from this balloon showed a rather abrupt rise when water which was swallowed reached its level. When a hiccup occurred there was a sudden and marked drop in the curve, due to an increase in the negative intrathoracic pressure. A pneumograph over the xiphoid process recorded the respirations on the tracing. No relationship was found between respiratory phases and the occurrence of hiccups.

The kymograph records showed that it required 0.7 to 0.8 second to pass water from the mouth into the esophagus, and that the hiccups

occurred within 0.5 second. Hiccups occurred before swallowing was complete. But it is possible that some water entered the esophagus before the occurrence of the hiccups. If we knew how far down the water passed before the hiccup occurs we could rule out, with reasonable assurance, irritation of structures below that level as the cause of the hiccup.

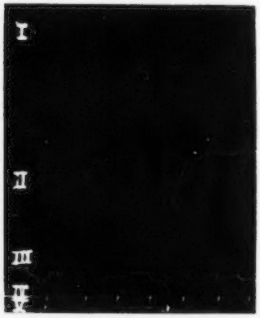

Fig. 1. A hiccup obtained on swallowing water at 62°C.

I. Respiration, recorded through a pneumograph over the epigastrium.

II. Intra-esophageal pressure, recorded through a balloon suspended in the esophagus about 7 cm. above the cardiac valve of the stomach.

III. Pharyngeal movements, recorded through a balloon fastened under the chin anterior to the hyoid bone. The curve showed a drop at the time the water passed backward in the mouth preparatory to swallowing. A rather quick rise occurred with the forceful part of swallowing—when the pharynx contracted, etc.

IV. Seconds.

V. Volition to swallow is recorded through pressure on an electrical key.

Subjectively, the hiccups seemed to occur so promptly on swallowing as to arise from irritation of the pharynx or of the esophagus immediately adjoining the pharynx. It felt as though the hot water did not enter the esophagus promptly on swallowing, possibly because of some spasm, and that because of such blocking the pharynx was momentarily subjected to an unusual stress. Repeatedly the sense of pharyngeal distention was almost instantly followed by a hiccup. But at other times (during meals) very small and quite deliberately taken swallows (or sips) of hot coffee or soup caused hiccups when there was surely no very great pharyngeal distention although there might possibly be a pharyngeal spasm. These hiccups have been a real annoyance at times. In two cases a hiccup occurred in a very abortive form on rapidly swallowing water at an ordinary drinking fountain. Carbonated drinks have caused hiccups. I find that a considerable number of other persons occasionally experience hiccups on drinking quite hot or otherwise irritating fluids.

Kymograph records (fig. 1) showed that water reached the balloon when suspended in the lower third of the esophagus (above the diaphragm) about 0.2 second before the occurrence of the hiccup. This indicated that the fluid might also have reached the level of the diaphragm before the hiccup had occurred. It is thought, though, that any fluid which might pass as far as the diaphragm level would by that time not be sufficiently hot to readily cause a hiccup by irritating diaphragmatic fibers because of its heat.

A second balloon was used in the esophagus, arranged similarly to the

first. The upper of the two balloons was used to impede the water until a hiccup had occurred. The lower balloon showed whether the upper balloon had properly obstructed the water. The lower balloon would also slow the passage of water. The occurrence of hiccups did not seem to be in any way altered by the presence of two balloons. Records showed that hiccups repeatedly occurred before any water had reached the level of the second balloon, which was always kept above the level of the diaphragm.

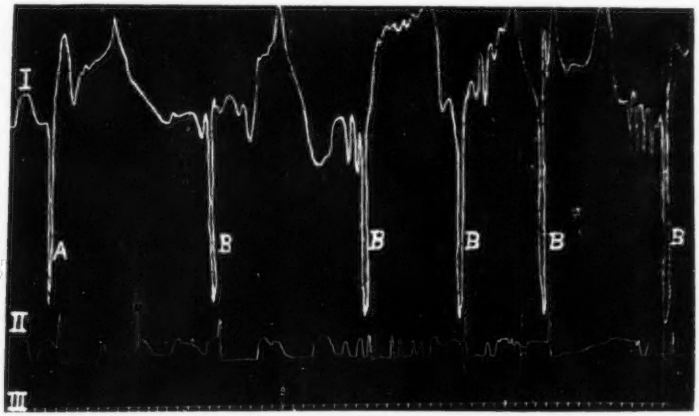

Fig. 2. Hiccups initiated by drinking water at 62°C. and sustained by irritation (of the esophagus) from an apparatus composed of two inflated balloons fastened together with wire and suspended in the esophagus. The upper of the two balloons was about 15 cm. from the cardia and the lower balloon 5 cm.

I. Intra-esophageal pressure recorded through the balloon located about 15 cm. from the cardia. *A*. Hiccup produced by drinking hot water. *B*. Hiccups occurring spontaneously.

II. Respiration recorded through a pneumograph over the epigastrium. The writing point for this curve was purposely out of alignment with the writing point above in order that the two might clear each other at the time of hiccups. (A more accurate alignment was used in figure 1.) The rate of respiration was varied to determine the effect on hiccups. The results were negative.

III. Seconds.

This shows that irritation of structures above such level will cause hiccups. This does not distinguish between pharyngeal and esophageal irritation.

Two balloons joined by wire such as to hold them 10 cm. apart from center to center were placed in the esophagus. When the further part of this combination was 5 cm. from the cardia there was a subdued sense of definitely localized esophageal irritation, with a slight desire to cough. It felt as though this irritation came from the wire where it was twisted at one end. When hot water was swallowed a series of 10 to 15 hiccups

occurred in place of a single hiccup as always occurred before and since in numerous trials by other methods using hot fluids. The hiccups were much more severe than those caused by hot water alone. I could neither anticipate the occurrence of the individual hiccups nor exert any voluntary control over them. When the hiccups stopped the sense of esophageal irritation with the desire to cough was gone. The apparatus may have shifted slightly so that the wire for some reason no longer irritated the esophagus. The balloons were moved until the sense of irritation occurred again. On swallowing hot water the preceding observations were repeated in their entirety. A part of the attack is shown in figure 2. It is concluded that the apparatus extending slightly below the middle third of the esophagus was not sufficiently irritating to start an attack of hiccups but that it did stimulate surrounding structures enough to sustain the attack when once started.

SUMMARY

1. On drinking water between 53° and 62°C. single hiccups occurred due to irritation of structures above the level of the diaphragm. Pharyngeal irritation seemed a most probable cause, allowing for irritation of adjoining structures.

2. A foreign body in the esophagus experimentally sustained an attack of hiccups.

3. It was found that an esophageal irritant capable of sustaining an attack of hiccups may not in itself be sufficient to initiate the attack.

This work was done under Dr. A. J. Carlson who gave a very essential aid and encouragement.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XXII. Blood Fat and Phosphorus in the Sexes and Their Variations in the Reproductive Cycle

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In an earlier paper of this series of studies Lawrence and Riddle (1916) found that the blood plasma of female fowls contains larger amounts of alcohol-soluble substance and of phosphorus than is found in males. Also that the hen whose reproductive activity is temporarily in abeyance contains less of these substances than is found in the actively reproducing hen. The data of Warner and Edmond (1917; see p. 292) for blood fat will be found to confirm both the sexual and reproductive differences in blood fat earlier observed by Lawrence and Riddle. In the study of Lawrence and Riddle it was pointed out that in various species of animals the sexes—as groups—were always, insofar as was known, thus characterized by a lower fat-content of the blood; that the male- and female-producing ova of pigeons had earlier been found by Riddle (1912) (1916) to be differentiated in the same sense; and that these facts probably reflect a primary—not a secondary—sexual difference. The present study indicates that the blood of male pigeons contains less fat than does the blood of females. The literature on this subject is reviewed at the close of this paper.

In the case of the fowl the activity of the ovary is of such nature as to preclude a study of blood changes which accompany each individual period of the growth and dehiscence (ovulation) of a single ovum or of a pair of ova. In the pigeon, however, this can readily be done; here the growth and ovulation of a pair of ova constitute a well-defined period in the reproductive cycle. Since important sexual phenomena have been shown by Whitman (1919) and by Riddle (1912) (1916) to be associated with the continuous recurrence of ovulation in the pigeon it is necessary to learn whether important blood and bodily changes occur in the female parent as a result of such continuous ovulation. In earlier papers of this series, such changes at ovulation—all indicative of notable metabolic change—have been described for suprarenal size, blood sugar and blood

calcium. Those studies showed that largest suprarenal size and highest concentrations of sugar and calcium are directly coincident with ovulation —increasing in pre-ovulation and decreasing in post-ovulation stages. The present study shows that the highest concentrations of blood fat and of lipoid phosphorus occur during the ovulation period. This demonstration of a cyclic change requires a review of the literature dealing with changes in blood fat and phosphorus in the reproductive cycle of animals, including man.

METHODS AND MATERIAL. It was desirable to estimate the amount of fat and the amount of alcohol-ether-extractable phosphorus on the same blood sample. In doing this we used Bloor's (1914) fat extraction methods, but determined the amount gravimetrically. The dry fat residue was used for a phosphorus determination by adaptations of the method of Stewart and Archibald (1925). A few cubic centimeters of carotid blood from a decapitated ring-dove were collected in a small beaker containing a little powdered sodium oxalate. Two cubic centimeters of this blood were pipetted into 80 cc. (100 cc. at end of filtration) of the alcohol-ether mixture and the extraction and filtration carried out according to Bloor. The alcohol-ether mixture was evaporated and the fat residue dried in vacuo and weighed.

For the phosphorus determination the weighed fat was softened with 1 cc. of distilled water, and another cubic centimeter was used in washing parts of it into a Jena test tube; this washing was completed with two or three successive additions of 1 cc. portions of the nitric-sulfuric mixture used for the digestion. From this point onward Stewart and Archibald's method for total phosphorus was largely followed—the titration (phenolphthalein) being carried out in CO_2-free air. Stewart and Archibald estimated 0.05 mgm. with an error of $\pm$ 3 per cent. Although we worked with notably larger quantities we were usually unable to obtain equal accuracy. Neither the unmodified molybdate precipitation nor the filtration through paper-pulp was found satisfactory. A very hard filter paper, and double filtrations and titrations were regularly resorted to in order that all the phosphorus might be obtained. This use of the hard filter paper made it necessary to wash the paper as free as possible from the molybdate precipitate before the paper itself was subdivided and dropped into the titrating vessel.

The animals used were blond ring doves (*Streptopelia risoria*) or later generations of its crosses with the white ring dove (*St. alba*). The exact age and the full reproductive history of each bird were known. Autopsies made at the time the blood samples were drawn permitted the recognition of most of the diseased birds, and enabled us to fix definitely the number of hours to the next ovulation for all females in "pre-ovulation" stages. The time relations for females in all other stages of the reproductive cycle

were known from the records available for each bird. Males are classed in the same reproductive stage as were their female mates. Only a few males were studied but usually these were brothers to their female mates and were killed on the same day. No period of fasting preceded the taking of any of the blood samples, since fasting would inevitably interrupt certain stages of the reproductive cycle. All that could be done was to draw the samples at approximately the same time of day (noon).

PRESENTATION OF DATA. The results are fully summarized in table 1; those for adult actively laying females, in normal stages of the reproductive cycle, are also given in the form of point-to-point curves in figure 1. We shall here use the term "fat" to designate the whole of the alcohol-ether-soluble substance of the blood and "lipoid phosphorus" for all that contained in this extract.

The natural relationships of the various parts or stages of the reproductive cycle of pigeons should be understood. The cycle is best thought of as beginning with a "resting stage"—having a duration of days or weeks—and ending with a period of "feeding young" (about 4 weeks). The interval between those end stages is occupied by four other distinct periods (three of them further subdivided in the table): *a*, the "pre-ovulation period" of 108 hours during which an ovum undergoes its final period of rapid growth; *b*, a mid-ovulation or "during ovulation" period of 44 hours—the interval separating the dehiscence of two fully grown ova from the ovary; *c*, the "post-ovulation period"—lasting about 108 hours before a return of the reproductive organs to a typical "resting stage" in birds not permitted to incubate; this latter merging after only about 60 hours into *d*, an "incubation period" of 15 days (ring doves). Females are considered "virgin" until they produce their first egg.

In the case of males it is possible that the "normal or resting period" should be thought of as lasting until the "incubation period" begins; but it is also possible that something of the nature of copulatory "exhaustion" immediately precedes the beginning of incubation by the male; it is certain, however, that already at or near the beginning of the "post-ovulation period" of his mate some bodily changes occur in the male which prepares him for incubation. These "bodily changes" involve a most profound change in his habits and activities; he then "sits" quietly on the eggs during most of the daylight hours and thus enters the most *inactive* period not only of the reproductive cycle but of his adult life cycle.

Sexual difference in blood fat. The blood of 9 healthy adult males, in the normal or "resting" stage, had an average of 1.77 grams of alcohol-ether soluble substance per 100 cc. (range = 1.59 to 1.98 grams); another such male, only two days before beginning incubation, had 1.76 grams; still another after only one day of incubation had a value of 1.60 grams. Only healthy adult females, in the normal or "resting" stage, are directly comparable with these males. The blood of 6 such adults had an average of 2.02 grams (range = 1.80 to 2.34 gms.); 4 virgins (two were plainly diseased; all were reproductively or sexually abnormal, having attained an age of 11 to 13 months without producing an egg) had an average of 1.81 grams; two additional healthy virgins (aged only 4.0 and 6.5 months)

which had received injections of "follicular hormone" about 5 to 2 days previous to sampling, had 1.93 grams. Possibly some significance attaches to the fact that the selected group of "virgin" females which had attained advanced age without having been able to function as females more closely approach the male values than does any other group; also that the female group which next most nearly approximates to male values is that of birds which had ceased laying for periods varying from 119 to 195 days.

It is thus found that two or three groups of males all had less blood fat than had any of the four groups of females just described. Indeed, all these male groups had less fat than had any of 11 additional groups of healthy females in other stages of the reproductive cycle; but it is clear that in at least some of these stages the blood fat values are not at all comparable even with the "resting" stage of females. Both the fat and phosphorus values obtained from diseased birds are too meagre and too variable to throw any light on the question of sexual difference. The data obtained from healthy birds seem sufficient to warrant the conclusion that among these ring doves the males have smaller amounts of blood fat than have the females.

Sex difference in lipoid phosphorus. Our data for males are entirely too meagre to permit any conclusion concerning this point. In view of the variability of the P values these are not reliably fixed by only two determinations, for males in the "resting" stage. The only other determination of this value in a male not plainly diseased was for an incubating bird of the most advanced age (32 months), having an enlarged liver, and with a fat value of (2.255 g.) 14 per cent higher than the highest found among 11 healthy males. This third phosphorus value was 38.5 mgm. per 100 cc. blood. Of course it may be that there is an increase of both fat and phosphorus in the "incubating" male, but on this point also the data are insufficient.

Fat and phosphorus changes during the reproductive cycle. The data are fully given in table 1. From the data for females the curves of figure 1 were constructed. These latter show that changes in the lipoid phosphorus run essentially parallel with the total fat. Since this is an expected result the parallelism actually obtained indicates the general trustworthiness of these average phosphorus values. The reliability of the figures for fat is scarcely questionable, but the number of phosphorus determinations is smaller and the individual determinations more variable.

The point of chief interest here lies in the fact that the highest values for blood fat and phosphorus do not coincide with a mid-ovulation period but precede it by 67 to 45 hours. As best shown by the curve these values rise early and rapidly in the pre-ovulation period; thereafter they decrease, though they remain above normal at the ovulation period; they attain a minimum or normal level toward the middle of the post-ovulation period,

TABLE 1

Blood fat (in grams per 100 cc.) and lipoid phosphorous (in milligrams per 100 cc.) in the sexes and in the reproductive cycle (ring doves).

SEX GROUPS	FAT AND LIPOID P	CEASED LAYING (MORE THAN 100 DAYS)		NORMAL OR RESTING STAGE		PRE-OVULATION PERIOD								DURING OVULATION		POST-OVULATION						INCUBATION				FEEDING YOUNG	
						88 hours		67 hours		45 hours		27 hours				16 hours after		45 hours		86 hours		4-9 days		10-15 days			
		Amount	Number	Amount	Number	Amount	Number	Amount	Number	Amount	Number	Amount	Number	Amount	Number	Amount	Number	Amount	Number	Amount	Number	Amount	Number	Amount	Number	Amount	Number
Adult females	Fat	1.83	2	2.02	6	2.26	11	2.59	4	2.73	4	2.29	5	2.32	9	1.97	5	1.85	5	2.12	3	2.23	5	1.95	4	2.12	4
	Lipoid P			36.8	5	45.8	7	56.6	2	49.6	3	40.8	4	42.2	8	38.5	4	38.2	5	39.7	3	44.9	5	38.2	1	37.1	3
Virgin females	Fat	1.93*	2	1.81*	4																						
	Lipoid P			(38.1)	2																						
Adult males	Fat			1.77	9							1.76	1			1.60	1					2.04	2				
	Lipoid P			39.7	2																	38.5	1				
Other[1] adult females	Fat	1.93	1	1.92	3	4.18	1																	2.34	1		
	Lipoid P																										
Other[2] adult males	Fat			1.95	3																						
	Lipoid P			51.3	1																						

* Virgins in resting stage; those in first column, of same stage but after injection with ovarian hormone.

[1] Diseased, or with yolks previously into body cavity.

[2] Diseased.

probably rise during early "incubation," and become essentially normal near the end of incubation and the period of "feeding young." This occurrence of a maximum change *early* in the pre-ovulation period is of considerable interest, since all of the hitherto observed changes (blood sugar, blood calcium, adrenal size; oviducal size, size of largest ovum) in the reproductive cycle of pigeons attain a maximum *later*—namely, coincident with the "ovulation period" itself. The known and suspected relations of the ovary to the metabolism of fats and lipoids, together with this early attainment of maximum values for these particular substances in the blood, suggest that it is the *ovary*—rather than other organs thus far studied (the adrenals, oviduct, parathyroids)—which supplies the *initiative* for the entire series of observed changes at reproduction. The dependence of the oviducal (uterine) growth upon the ovary is already well-known. Of course it may well be that the real or ultimate initiators are factors

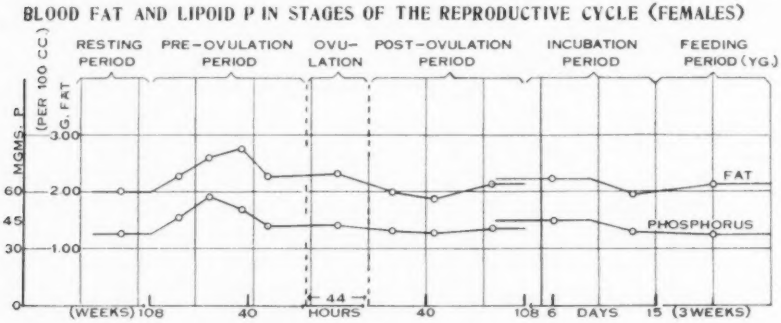

Fig. 1

hitherto unstudied; and indeed some recent work on the anterior lobe of the pituitary would indicate that that organ primarily activates the ovary.

In table 2 we give a summary of certain points in the present and previous data bearing on the fat and phosphorus content of blood, plasma or serum of birds (fowl and pigeon). Weiske (1889) found the total P_2O_5 of five "fowls" to range between 1.55 and 1.8 per cent of the blood solids. The tabulated data make clearer the result that in birds thus far studied[1] the blood fat is present in smaller amounts in the male than in the female. They further show that the fat content of the blood of the non-laying fowl, and of the pigeon in the "resting stage," is less than that of the laying fowl or pigeon. The lipoid phosphorus of fowls shows a fairly similar difference; males having least, laying females most, and non-laying females

[1] We know that some determinations on the blood fat of pigeons have been made by Japanese workers (Iwatsura; Ogata) but their papers are unavailable and are apparently written only in Japanese.

an intermediate amount. The same holds true as between the "resting" and "ovulation" stages of the female pigeon, while male values for the pigeon are thus far inadequately studied. The data of Lawrence and Riddle are here for the first time calculated in terms of per cent of whole plasma. Their published calculation was based on percentage of plasma solids only.

DISCUSSION. The results recorded here permit a further examination of the question of the differentiation of blood fat in the sexes. They also

TABLE 2

Summary comparison of percentage "fat," and of alcohol-ether-extractable phosphorus (in milligrams), in the blood of fowls and doves

BIRD	OBSERVERS	FAT OR P	MALES	NON-LAYING	LAYING, OR MATURING EGGS	DESCRIPTION OF VALUES GIVEN
Fowl	Hammarsten*	Fat			1.45*	Per cent, in serum
	Lawrence and Riddle† (1916)	Fat	0.783	1.076	1.687	Per cent, in plasma
		P	40.1	42.7	73.1	Milligram per 100 gram plasma
	Warner (1916)	Fat			1.426	Per cent, whole blood
	Warner and Edmond (1917)	Fat	0.176	0.192‡	1.109	Per cent, whole blood
Dove	(Present work)	Fat	1.77	2.02	2.73	Per cent, whole blood
		P	(39.7)§	36.8	49.6	Milligram per 100 cc. blood

* According to Ingle (Manual of Agr. Chem., 1913). The original work not seen and the high figure for fat is the only reason for our classification as a "laying hen."

† The figures published were in an unfortunate and incomplete form (per cent of *solids*, and P as lecithin). They are here recalculated to fall into the categories indicated in the heading of this table.

‡ The authors give two groups: One (of 12) for 3-year old hens, value 0.171; another (of 54) undescribed, value 0.199. The total for all non-layers is 0.192 (see text).

§ An unreliable figure (average of only two dissimilar values).

throw some light on the series of bodily changes which accompany the reproductive cycle in pigeons. The bearing of our results on the latter problem will be considered first, though it may be preceded by two general statements.

In a previous paper (Riddle, 1923) curves were constructed showing that at or near 108 hours preceding an ovulation, the suprarenals begin to increase in weight, an ovum begins an increased rate of growth, and the oviduct begins a phenomenal increase in size. The ovum attains its maximum size at ovulation, and both suprarenal and oviduct attain their

maximum size during ovulation—that is, within the 44-hour interval between the dehiscence, or ovulation, of the *two* ova produced by pigeons. In a later paper Riddle and Honeywell (1923) showed that the blood sugar reaches a maximum value at this same time. Still later, Riddle and Reinhart (1926) found that the blood calcium is doubled in value and also attains its maximum during the ovulation period. In comparison with all of the changes just mentioned we now find that the blood fat and lipoid phosphorus also increase during the ovulation cycle, but the latter values differ from all the others in reaching their maximum *earlier*, namely, in the pre-ovulation period. We have already referred to the possible bearing of this fact on the difficult task of identification of the primary source of the whole series of changes which accompany an ovulation cycle.

A relation of the ovary to fat and phosphorus metabolism in general, is widely recognized. Women store fat after the removal of the ovaries, or after destruction of the ovary from either of various causes, and after the menopause. Some intimate connection of the ovary with the abnormal phosphorus utilization involved in cases of osteomalacia is also well-known. While it is possible that a removal of the ovary may only indirectly affect the metabolism of fat and phosphorus it is nevertheless notable that such castration does not produce equivalent effects upon the metabolism of protein or carbohydrate. By castration experiments, and by the injection of ovarian extracts, Hisaw (1925) has recently demonstrated the normal relation of the ovary to the dissolution of the pubic symphyses in the pocket gopher.

On the other hand, the following discussion will show how incomplete and uncertain are the data concerning the cyclic changes of ovarian action to either blood fat or to lipoid phosphorus in man and mammals.

Blood fat and phosphorus in the reproductive cycle. The changes of blood fat in the menstruation cycle in women have been very little studied. Goñalons (1917) reported on 27 cycles in which samples were taken each second day for cholesterol determinations. Two types of curves were found. In the most common type the cholesterin began an abrupt rise 2 to 7 days before menstruation and continued to near the end of this period. In the other (4 cases) the increase occurred but the rise was gradual. From two castrated women he obtained notably high cholesterol values. Moynihan (1925) partially reported upon the changes in cholesterol during the cycle. He finds immediately before and during the first day or two of menstruation a high value quickly followed by a fall. A minimum coincides with the end of the menstrual period, and this is followed by a somewhat higher level of short duration; for two weeks thereafter a normal level is obtained. If ovulation occurs in woman between the 10th and 16th days after the beginning of menstruation it would thus seem that *at ovulation* the cholesterol value is probably normal, and that an increased

cholesterol value precedes it by as much as 10 or more days. The published data, however, are not very convincing.

While this paper was being written a study of changes in blood fat during the menstruation cycle was published by Okey and Boyden (1927). In this work cholesterol and fatty acids were separately determined, and lecithin was calculated from the phosphorus values. Twenty-six periods in 16 healthy subjects were studied. Unfortunately they give no figures for "total fat" and these are not calculable from the tabulated values; their detailed summaries of cyclic changes deal with cholesterol only. These authors conclude: "The most striking and consistent cyclic alteration in blood lipid content observed was the fall in blood cholesterol, which took place almost invariably during or within a few days of the menstrual period. This was usually preceded or followed by blood cholesterol levels higher than the averages for the individuals concerned." To a considerable extent these data seem to us to confirm the work cited above concerning an increased cholesterol level coincident with menstruation. The data of Okey and Boyden well support their further conclusion that "the blood cholesterol level in women is to be considered as a variable rather than a constant," that the "lecithin" value remains practically constant, and that the "fatty acids" do not consistently vary during the cycle. Our own separation of their data for the cycle into five periods indicates that periods near and during menstruation average slightly (probably insignificantly) higher (407, 409, 404) than the periods most removed from it (384, 387). If ovulation in the human bears any constant relation to menstruation these data give little or no suggestion that there are cyclic changes in the human similar to those we now find in the pigeon.

In some mammals the total blood fat is known to increase during pregnancy. This was first found in the dog by Capaldi (1904). On the other hand, Baumann and Holly (1926) found "that a progressive decrease in the cholesterol and phosphatide content of blood takes place during pregnancy in normal rabbits, beginning 7 to 10 days after conception and reaching its minimum a few days before or at parturition." Moreover in only one of three dogs did they find a definite rise in blood cholesterol and phosphatides; and this occurred for only about 10 days—just before and after parturition. Again, Meigs, Blatherwick and Cary (1919) obtained some evidence that in the cow the lipoid and total blood phosphorus decrease near the end of pregnancy. These workers find that lactation is a period of increased concentration of fat and phosphatid in the cow's blood. The increase in blood fat during pregnancy in woman seems to be well demonstrated; it involves the several fractions—cholesterol, cholesterol esters and lecithin—and is graduated to a highest point at term. The literature has been recently discussed in the contribution of Tyler and Underhill (1925).

A few observations on the blood or hemolymph of lower animals are available. Miescher (1897) found that at the time of ripening eggs in the salmon the blood serum contained "erhebliche Mengen" of lecithin and fatty acids. Heim (1892) showed that in a number of species of Decapod Crustacea the blood acquires a brilliant yellow lipochrome (alcohol-

soluble) pigment at the time the ovaries mature and function. In birds the evidence for an increase of blood fat associated with ovulation or egg-laying is uniform, adequate and convincing. Lawrence and Riddle (1916) first obtained evidence of this in the fowl. It was independently found by Warner (1916), and later confirmed by Warner and Edmond (1917; see also Riddle and Harris, 1918). The present study demonstrates the occurrence of a less striking though unmistakable increase in the pigeon.

Blood fat as a sex-differential. Gorup-Besanez (1878) makes the meagre but interesting statement in some tabulated data that the blood of males contains *less* fat, that of females *more*. Some data on the blood fat of men and women by Gettler and Baker (1916) were tabulated by Lawrence and Riddle and shown to indicate higher average values in the five female bloods than in the males; also the two females with presumably functional ovaries (30 and 35 years old) gave higher values than was found for the three women of advanced age (48 to 59 years). Bloor (1916) determined separately the various lipoids of the blood in 14 normal males and 7 normal females. The lecithin values were nearly equal (0.30 to 0.29 g. per 100 cc.); the total ether-soluble (fat) values slightly less in the males (0.67 to 0.72). Bloor concluded that "the differences in the content of lipoids of men's and women's blood is not great, and it is possible that a more extended series of analyses may eliminate these differences."

Jones and Nye (1921) determined the total amount of phosphorus in the whole blood and in the lipoid fraction of whole blood in 14 boys and 17 girls aged 4 weeks to 14 years. Some of these were convalescent hospital cases. The averages obtained for whole blood of 14 boys were 123.3 mgm. H_2PO_4 per 100 cc. for boys and 121.2 mgm. for girls. Since the individual values varied in boys between 88 and 150, and from 90 to 150 in girls it is evident that the difference obtained is not significant. For lipoid phosphorus a larger difference was found, the averages being 44.5 (31 to 50.2) for boys, and 38.4 (25 to 51.5) for the girls. Again, the significance of these figures is very questionable. McKellips, DeYoung and Bloor (1921) made similar determinations for the various forms of phosphorus in the plasma and corpuscles of infant boys and girls. The lipoid phosphorus of the plasma averaged 15.0 mgm. (10.1 to 18.2) H_2PO_4 per 100 cc. in 10 boys; 14.1 mgm. (10.4 to 20.8) in 11 girls. Apparently more striking differences, with lower inorganic values (9.0♂ : 11.2♀) in the plasma of males and with higher organic values (170.3♂ : 147.6♀) in the corpuscles of males, were found. The authors attach little or no significance to these differences.

From some invertebrates evidence of a much more definite nature has been obtained concerning a sexual differentiation of the fat content of the blood or hemolymph. G. Smith (1911) showed that in female crabs whose eggs are maturing the blood is much richer in fat than is the male blood; and—of greater importance—that the blood fat is much increased in male crabs which undergo partial sex-reversal (toward femaleness) under parasitic castration by *Sacculina*. In certain moths Steche (1912) and Geyer (1913) showed that the alcohol-soluble pigments differ both

quantitatively and qualitatively in the sexes. The blood of the females contains both the chlorophyllin and xanthophyl present in the leaves on which it feeds; their blood is therefore greenish. In the blood of males, however, the chlorophyllin is not present, and the xanthophyl is sometimes present and sometimes not; their blood is therefore yellow or colorless. It is not impossible that quantitative differences in alcohol-soluble substances (cholesterin, lecithin) may be responsible for the precipitin reactions which led these authors to conclude that they had also demonstrated sex-specific proteins in the blood of these insects. In support of the type of change observed by Smith is Kornhauser's (1919) observation that in parasitized *Thelia* the total fat value of the body of males assuming female secondary sex characters is raised (chemical analyses by O. Riddle) from 41 per cent to nearly 49 per cent. In some of the sporozoa Joyet-Lavergne (1925) has shown by microchemical tests that the females contain greater relative amounts of lipoid granules than do the males.

Though the number of determinations is not large the few investigations that have been made on the blood fat of birds show, practically uniformly, that male blood contains less fat than female blood. Only non-laying females are comparable with males and these data are adequately summarized in table 2. Inspection of that table, however, raises a question as to the accuracy of one or the other series of determinations of blood fat in male and non-laying fowls. These values as found by Warner and Edmond are only about one-fifth of those of Lawrence and Riddle, and it is obvious that with so wide a discrepancy (though one is for whole blood, the other for plasma) both series can hardly be used as evidence for blood fat as a sex-differential. In estimating the accuracy of the two series we may note as follows: Lawrence and Riddle used much larger samples (20 to 112 grams instead of 5 cc.). Their samples were quickly submitted to a temperature (85°C.) which probably destroyed the esterases present though it would permit some oxidation of the fats; they then extracted with both alcohol and ether. Warner and Edmond dried their samples over phosphorus pentoxide *in vacuo* at 50 to 60°C., and extracted with ether. In general, these considerations probably favor the values obtained by Lawrence and Riddle. Moreover, the lipoid phosphorus determinations carried out by the latter bear a fairly similar ratio to the amount of fat found in their entire series of determinations. Again, their values for fat in the male and non-laying female fowl much more nearly approach the values which we now find in the pigeon. Though Warner and Edmond made many more determinations than did Lawrence and Riddle they made no autopsies on any of their birds in order to learn whether they were healthy or diseased. In both series of determinations on the fowl, and more particularly in that of Lawrence and Riddle, the male fowls appear to have least fat in the blood. In the case of the pigeon the males as a

group are clearly differentiated from the group of "resting" females by a smaller amount of blood fat. Adequate studies on the phosphorus content of the blood have been made neither in the fowl nor in the pigeon.

Our examination of the literature for blood fat in the human subject, both on the question of changes in the reproductive cycle and of a possible sex difference, gives rise to the following observations: The available data indicate that a sex difference, if present, is small in the human; and that, though a rather wide variability in total fats and of the individual lipoids is indicated within the reproductive cycle, there is little consistent variability detectable. For the measurement of these small differences and for this inconsistent variability are our control of conditions and our methods of analysis adequate? The basal metabolism of men and women is small—only about 6 per cent—and it could be satisfactorily demonstrated only after a control of conditions considerably more rigorous than is used in the drawing of blood samples for estimation of their fat content. Further, the small samples usually taken from the human subject involve an error that becomes important if *small* differences are to be detected. These observations apply also to studies of variability during the sexual cycle. To us it seems probable that definite knowledge of these differences in the human requires the use of larger blood samples and a hitherto unpracticed degree of control over various conditions which may be found to affect the level of blood fat in the donor.

The available data make it fairly evident that in both of the species of birds thus far studied the females have a higher percentage of alcohol-ether-soluble substance in the blood than have the males. For the fowl more data are desirable; for the pigeon further data may not be expected to change the result. This latter is the same species in which Riddle has earlier found a similar differentiation to characterize the two kinds of ova—the male-producing and the female-producing ova. The type of difference with respect to fat content which was earlier found in the egg-stage is here shown to persist in the blood of the two sexes. Where data for blood fat are available for other forms than birds—even in invertebrates—there also some uncontradicted evidence is found that these substances are present in greater amounts in the female. This widely distributed quantitative difference is probably to be regarded not as a secondary sex character, but as a direct reflection or accompaniment of the metabolic difference which one of us has previously identified as the primary dissimilarity of the sexes.

SUMMARY

During the ovulation cycle of female ring-doves the amount of alcohol-ether-soluble substance in the whole blood is increased to 35 per cent above the normal or "resting" value. The phosphorus contained in this extract is increased to approximately 50 per cent above the normal value.

This well-marked cyclic increase in the fat and lipoid phosphorus of the blood recurs with each ovulation period. In female doves forced to continuous egg-production the metabolism of fat and phosphorus is therefore repeatedly brought to and temporarily maintained at an unusual level. The literature on changes in blood fat and phosphorus in the reproductive cycle in animals and man is reviewed.

Cyclic changes earlier observed in these doves—suprarenal hypertrophy, increased blood sugar, increased blood calcium—all reached a maximum coincident with the "ovulation period." The blood fat and phosphorus, however, attain their maximum 67 to 45 hours earlier—in the pre-ovulation stage of the cycle. Because of the known relations of the ovary to fat metabolism this earlier maximum of fat suggests that the ovary itself—rather than suprarenals or parathyroids—is more directly responsible for the entire series of changes hitherto observed in the reproductive cycle.

Considered as groups normal male ring-doves have less blood fat (1.77 g. per 100 cc.) than normal females (2.02 g.) in the "resting stage" or in any other stage of the reproductive cycle. An increased storage of fat earlier found by Riddle to characterize the female-producing ova of pigeons is thus paralleled by a higher concentration of fat in the blood of adult females.

The literature dealing with blood fat as a sex-differential is reviewed. From the protozoa to man there is meagre but uncontradictory evidence that the metabolism of fat is not equal in male and female. This inequality is probably not to be regarded as a secondary sex character, but as a direct expression of the metabolic difference which one of us has earlier identified with primary sex difference.

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OBSERVATIONS ON THE ACTIVITY AND WORKING POWER OF THE UTERINE MUSCLE OF THE NON-PREGNANT SOW[1]

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This paper contains a record of observations on the activity, with reference to the oestrous cycle, of the non-pregnant uterus of the sow. It confirms and extends Keye's work (1) on the character of the contractions and gives measurements of the working power of the uterine muscle.

The literature on the movements of the uterus has grown rapidly during the last twenty years but, since Guggisberg in a recent article (2) has included an extensive review of it, attention will be called only to work bearing on special phases of the immediate problem. The pregnant and non-pregnant uteri of various laboratory animals as well as of the human show a remarkable degree of spontaneous activity. Sun (3) in the light of his own observations and those of others and from analogous conditions in animals, feels justified in concluding that the human uterus contracts rhythmically in the 6 months' fetus, through all periods of gestation and until after the menopause. It was brought out by Cushny (4) and others that the contractions of the excised uterus of the young virgin are more regular and less active than those of the mature animal, a significant matter, when the uterus is used as an indicator for testing the activity of certain drugs, as Dale and Laidlaw (5) and Smith and McClosky (6) have shown for pituitary extracts. It was not, however, appreciated that the variability of the spontaneous contractions is dependent on the stage of the oestrous cycle, until Blair (7) demonstrated it for the rat in 1922. Independently, at about the same time, Keye reported that changes in the activity of the isolated uterine rings of the pig accompanied histological changes in the ovary and endometrium.

These were followed by the observations of Clarke, Knaus and Parker (8) on the rat's uterus *in situ* and of Wislocki and Guttmacher (9) on the entire excised uterus of the sow. Their papers gave important informa-

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tion regarding the origin and conduction of the contractions. Such studies would not have been possible had not the work of Long and Evans (10) on the rat, and Corner (11) on the pig, made it a fairly simple matter to follow the stages of the oestrous cycle.

Another phase of the problem of uterine activity which has attracted attention is the difference in activity of the longitudinal and circular muscles. These layers are sharply defined in the uteri of laboratory animals. Cushny (4) found their contraction in the dog, cat and rabbit very similar in character, though not simultaneous, when he registered the movements of the uteri *in situ*. In a number of experiments there were strong, long-continued contractions of the circular coat, such as were seldom seen in the longitudinal. Sometimes contractions of the circular masked those of the longitudinal muscle. Ogata (12) gives simultaneous tracings of strips and rings of the virgin rabbit's uterus in which the longitudinal layer was contracting vigorously and the circular scarcely at all. Wislocki and Guttmacher (9) mention the predominance of the peristaltic type of contraction and the absence, as a rule, of definite contraction rings.

Still another significant problem regards the working power of the uterine muscle. Attempts have been made to determine the work done by the contractions of the human uterus during labor. Guggisberg (2) and Bigler (13) do not regard the results obtained by the methods used as of more than theoretical interest, since they are a measure of the force exerted and not of the actual working power of the uterus. They devised a manometer method, by the use of which they were able to measure the work done by the freshly excised and emptied uterus of the pregnant guinea pig. They found it to vary in one horn from 4 gm. cm. to 32 gm. cm. Some unpublished investigations from this laboratory have compared the working power by the use of the work-adder of the guinea pig's uterus with reference to the phase of the cycle. I am not aware of any other attempt to estimate the working power of the non-pregnant uterus.

MATERIAL AND METHODS. For a number of reasons the sow's uterus offers unusual advantages for the study of the working power of the muscle. Abundant material is available at all seasons of the year from a large abattoir; the place of the specimens in the 21 day oestrus cycle can be accurately determined by the histological method; further, the isolated muscle from an active specimen will continue to contract rhythmically for two or three hours and is less easily inhibited than the uterus of the guinea pig. On the other hand it is impossible to have the careful control over the material, from the moment the pig is killed, which is possible with the small laboratory animals. No doubt the uterus is often subjected to rough handling at the slaughter-house and this may account for the inactivity of some specimens. In this series, because of the distance, a half-hour of necessity elapsed before the material reached the laboratory.

The observations extended from July until January, and were made on a series of 103 non-pregnant uteri. A number of records for one reason or another were discarded, so that this report is based on 70 specimens. The working power was recorded for 55 of the 103. During the period, 36 pregnant specimens were also studied but, as this series is being extended, the results will be reported later.

The uteri were stored as soon as they reached the laboratory and a piece of the ovary and of the uterus of each specimen selected was placed in Bouin's fixing-fluid. If the gross appearance of the corpora lutea indicated oestrus or the four days following, the Fallopian tube or uterus was washed out by the method described by Corner (10) and the washings searched for ova. Since it was possible to carry out a physiological study on only one specimen at a time, the others were immediately placed in the refrigerator. A few hours or even twenty-four did not apparently interfere with their activity. In fact, in a number of cases, inactive muscle became very active after such treatment. Contractility was feeble and irregular in those tested on the third day. This is an interesting contrast to the prolonged activity of the pregnant human uterine muscle reported on by Sun (2).

Longitudinal and circular strips measuring 3 cm. long and 0.5 cm. broad, before an incision was made, were cut from the uterus between 30 to 50 cm. from the tubal end. They were tied to oxygen tubes and immersed in standard Locke's solution at 37.5°C. (Sometimes they were placed in the cold solution. The only disadvantage I could see was that activity was delayed.) The Locke's solution was prepared with all the precautions, as indicated by Seckinger and Snyder (14), which work in this and other laboratories has shown to be essential in order to obtain uniform results with plain muscle.

The water-bath, electrically heated and automatically regulated, kept a temperature of 37.5°C. It had the special advantage of being large enough to hold several containers at one time. The thread from the muscle was attached to the hook on a straw held in a "straw-holder" (C. F. Palmer, London), the arms of the lever being 22.5 cm. and 4 cm. This lever was exceedingly light and the glass writing pointer, of the Sherrington type, worked with the minimum degree of friction. A longitudinal strip, like the one described, taken from the same part of the uterus, was set up at the same time for a study of the working power. For this measurement, a modification of the Fick work-adder, manufactured by the Harvard Apparatus Company and graduated in 0.1 cm. in the laboratory, was used. The thread from the submerged muscle passed over a pulley and then to the arm of the work-adder. Since a graphic record was desired, a straw with a glass writing point was attached, the lever arms being 24.7 cm. and 21 cm. long. The weights were suspended to a hook just below the attachment of the muscle thread.

Early in the course of the investigation it was found that strips of circular muscle, such as Keye used, were very inactive when attached to the work-adder, even though they showed rhythmical contractions on the light lever. On this account all the work records were made from longitudinal muscle. It is desirable, before discussing the working power of the muscle at various stages of the cycle, to consider the differences in the activity of the two layers of muscle. Thirty-three of the 70 specimens were taken during oestrus. Of these 14 were dated from 18 to 21 days after the last ovulation when the follicles are maturing and 19 from 1 to 3 days, when the ova are in the tubes. In 12 specimens taken 4 to 7 days after ovulation, ova were found in the washings from the uterus. Twenty-five belonged to the interoestrous period. (Both circular and longitudinal muscle were not used in every case.) In several cases, simultaneous tracings were taken of the two layers, with the specimens in the same vessel of Locke's solution. Such interesting differences in the character of the contractions were observed that, in a representative number of subsequent specimens, the circular and longitudinal muscles were separated, suspended in a similar volume of the same Locke's solution as the unseparated muscles, and simultaneous contractions recorded for them at the same time.

A consideration of these records may conveniently begin with the type found at the onset of oestrus. Figures 1 and 2 are taken as typical of the period. The ovary of this specimen (no. 124) contained 10 mm. follicles, corpora lutea of the last ovulation measuring 5 mm., and an older set 2 mm. in diameter. The circumference of the uterus was 44 mm. The histological examination of the ovary and uterus placed the date at 21 days after the last ovulation. The specimen was left in the refrigerator 17.5 hours. Both sets of muscle strips were relatively inactive for 45 minutes and the contractions did not become regularly rhythmic until nearly an hour later. Figures 1 and 2 are from the second hour of activity. The major and minor contractions described by Keye are evident in both layers of unseparated and separated muscle. In the former, some of the major contractions have a slightly longer duration in the circular than in the longitudinal layer, so that there are 22 of them in an hour to 25 of the longitudinal contractions. The minor contractions are less abrupt in the circular. The contractions of separate layers have a briefer duration and they occur half as frequently as the unseparated. They are more alike, except for amplitude, than those shown in figure 1.

After the follicles had ruptured and ova were found in the tubes, the character of the contractions was the same; contrary to Keye's statement, minor contractions were always present. In late oestrus, especially if the ovary contained an excessive amount of lutein tissue, the contractions had the appearance of a transition between the oestrus and post-oestrous type.

Fig. 1. Contractions of the unseparated longitudinal and circular muscle of the uterus of 124, 21 days after ovulation. The ovary contained 10 mm. follicles. The higher contractions are from the longitudinal layer. Time in all records 1 per minute.

Fig. 2. Contractions of the separated layers from the same specimen as figure 1. The higher contractions are from the longitudinal muscle.

Fig. 3. Contractions of the unseparated longitudinal and circular muscle of the uterus of 131, 2 to 3 days after ovulation. The ova were found in the tubes. The higher contractions are from the longitudinal muscle.

Fig. 4. Contractions of the separated layers from the same uterus as the record in figure 3. The higher contractions are from the longitudinal muscle.

This is illustrated by figures 3 and 4 from no. 131. Here the ovary contained recent corpora lutea 5 to 6 mm. in diameter, hemorrhagic but with the points of rupture closed. Corpora of the preceding ovulation measured 4 mm. Ten ova were recovered from one tube and 6 from the other. The histological examination indicated ovulation 2 to 3 days before. The muscle strips were taken while the uterus was still warm. There is greater irregularity in the activity of both muscles before separation than in num-

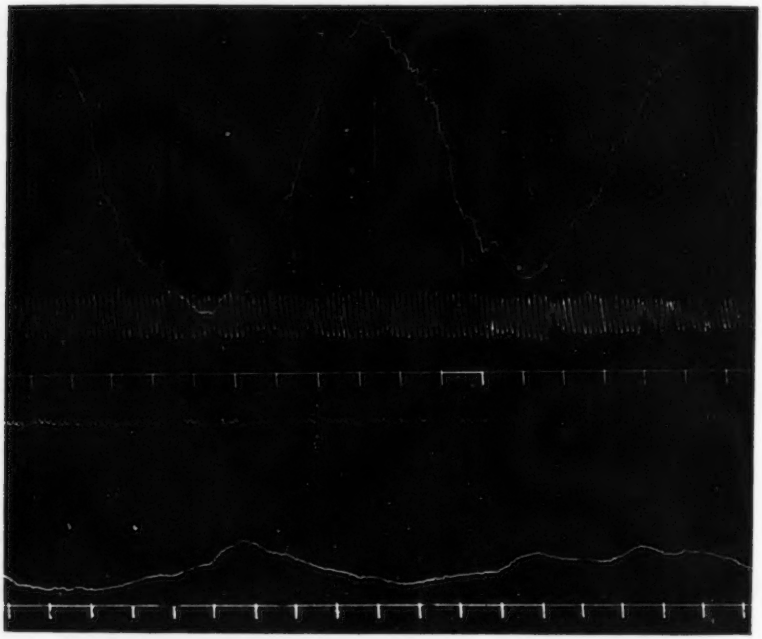


Fig. 5. The uppermost curve is from the separated longitudinal muscle of the uterus of 137, taken 7 days after the last ovulation, when the ova were degenerating in the uterus. The next curve below is from the separated circular layer, the third from the unseparated circular and the lowermost one from the unseparated longitudinal muscle.

ber 124, and the minor contractions become much more pronounced in the longitudinal, so that they resemble periods of incomplete tetanus, 3 to 5 minutes in duration. The circular layer gives small irregular major contractions with superimposed minors. Perhaps the unseparated layers interfere with one another's activity, for when dissected apart, they show much greater regularity both in rate and duration, this is especially marked with the circular muscle, which has greatly increased in amplitude and

contracts almost twice as rapidly as the longitudinal. The minor contractions have a 2, 3, 4 rhythm.

A record from the muscles of no. 39, dated 5 days after ovulation, when the ova are in the uterus, shows a similar type for the circular layer, but the longitudinal layer is slower, and the minor contractions less pronounced. About the seventh day (fig. 5) a change is evident. The ovary of this specimen (no. 137) contained recent corpora 7 mm. in diameter and those of a previous ovulation 4 mm. The uterus had a diameter of 40 mm. The unseparated longitudinal muscle shows undulating waves of small amplitude, lasting 6 to 7 minutes, with superimposed minor contractions, and the circular layer has a rate of 7 per minute. Separating the layers makes no essential difference in the character of the contraction but increases the amplitude.

By the tenth day the corpora lutea are at the height of their development and in no.129 (figs. 6 and 7) measured 8 to 10 mm. in diameter; those of the preceding ovulation 4 mm. The records were made after the specimen had been in the refrigerator 17 hours. The rate of the circular muscle continues to be from 7 to 8 per minute and the other is characterized by irregular tonic periods with slow superimposed contractions. Again separation increases the amplitude of contraction and there is a suggestion of tone in the circular layer. At this stage the records are more nearly comparable to those of a large number from pregnant uteri covering the first half of gestation.

Retrogression of the corpus sets in about the 15th day. By the 16th to the 17th day the corpora have a yellowish tinge and have decreased to 7 or 8 mm. in diameter. Accompanying this degeneration, there is a gradual change in the activity of the muscle. As if some stimulating agent were removed, the circular muscle has slowed down to 3 or 4 contractions per minute but they begin to have the oestrous character. On separation the amplitude increases in both layers and the secondary contractions are so pronounced as to give the longitudinal contractions the character of incomplete tetanus.

As the follicles rapidly increase, by the seventeenth day (no. 138) they have attained a diameter of 6 to 7 mm. and the retrogressing corpora 5 to 6 mm. Records were taken as soon as the specimen was received (figs. 8 and 9). The longitudinal muscle shows regular vigorous contractions of 3 to 4 minutes' duration and the circular has approximately the same rhythm with small superimposed waves. When separated, the inner muscle acts very much as it does three days later, its rate averaging 2 per minute, three times that of the longitudinal. Further observations of intermediate stages have made even more clear how, within the next two days, the uterine muscle assumes the character already described for the oestrous period. Sometimes this type appears when the surface diameter of the follicles is but 6 to 7 mm.

WORKING POWER OF THE MUSCLE. The natural inference, on the basis of the preceding observations, would be that the working power of the uterine muscle is greatest during the oestrous phase, and this is supported by the records. To measure its activity, the muscle was attached to the

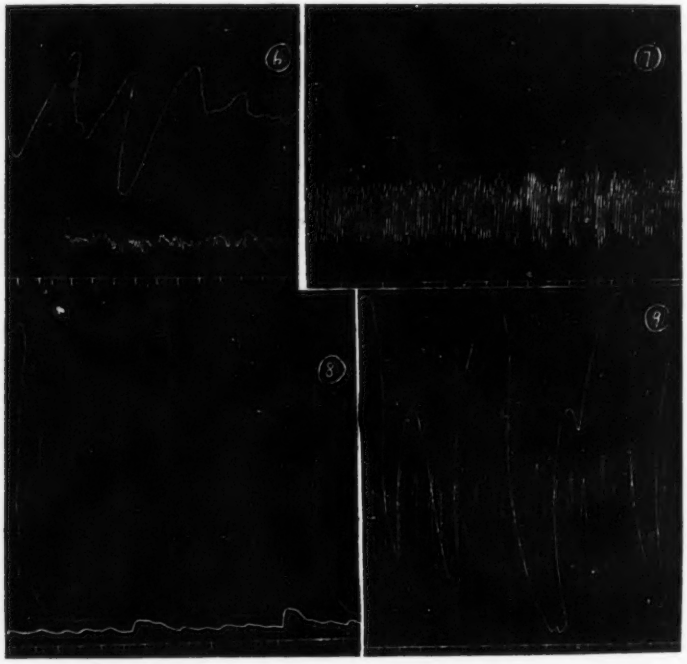


Fig. 6. Contractions of the unseparated longitudinal and circular muscle of the uterus of 129, 10 days after ovulation. The ovary contains 8 to 10 mm. corpora lutea. The upper curve is of the longitudinal muscle.

Fig. 7. Contractions of the separated layers from the same uterus as figure 6. The record of the longitudinal muscle is above.

Fig. 8. Contractions of the unseparated longitudinal and circular layers of the uterus of 138, 16 days after ovulation. The ovary has 6 to 7 mm. follicles. The upper curve is from the longitudinal muscle.

Fig. 9. Contractions of the separated layers of the same specimen as figure 8. The higher curve is from the longitudinal muscle.

lever of the work-adder, under a tension equivalent to 3 grams. The lever was not weighted until the muscle contracted to its maximum, turning the dial of the work-adder through 2 cm. This would occur within 45 minutes in an active muscle. If the specimen had been in the refrigerator, a longer time was needed in order that it might reach the

temperature of the Locke's solution. During a quiescent stage the extent of contraction was less and was reached more slowly. A fresh muscle which continued inactive for more than an hour was discarded, since such specimens were found to have little working power. When the maximum degree of contraction was reached, weights were added, beginning with 3 grams for a muscle in an active phase and with 1 or 2 grams for the inactive. After the muscle had contracted under this load for at least 15 minutes, the weight was removed and an interval allowed for it to shorten again, when unimpeded. This might require 5 minutes or 15. The weight was then increased to 5 grams, then to 10 and so by 5 gram stages, until the muscle was no longer able to record its contraction. For the less active specimen, the increase in weights was made more gradually. In order to compare the working power of the different muscles, the total quantity of the work done was determined in gram-centimeters and this reduced to gram-centimeters per minute.

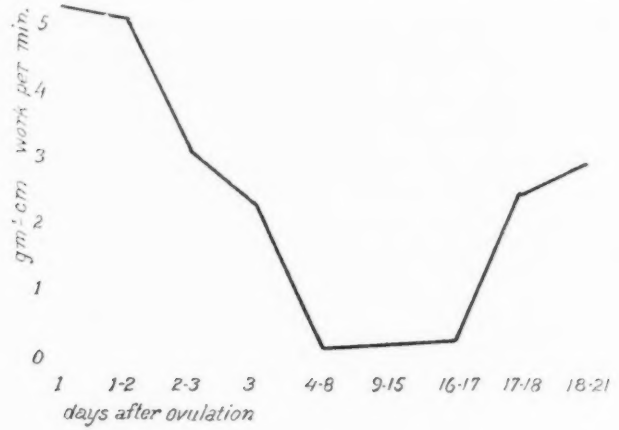
Work records were taken from 15 specimens of uteri in the oestrous stage, dated from 1 to 3 days after ovulation. Those of the first two days showed greatest activity, the maximum amount of work being performed by no. 8, 664 gm.cm. in 2 hours and 10 minutes, or 5.1 gm.cm. per minute. This muscle contracted when weighted with 40 grams. Some specimens in this series, which contracted in a typical manner on the unweighted lever, were very inactive when required to lift a weight.

A surprising change in the working power takes place when the ova pass into the uterus, 4 days after ovulation. The work performed was determined in 6 such cases and in not one of them did it exceed 0.3 gm.cm. per minute in contrast to an average of 3.3 for the period of oestrus.

The low value continues after the ova have degenerated and until the 17th day. On the basis of the records for 19 specimens the work done was found to be slightly more than in the preceding stage but, even so, the greatest amount was only 160 gm.cm., 0.9 per minute. From the fourth to the fifteenth day the corpus luteum has rapidly increased to its maximum size. It is at this period that the contractions of the unseparated layer of longitudinal muscle are slow, irregular, and of small amplitude. Retrogression of the corpus begins by the 15th day. On the 17th or 18th day there is again found to be an increase in the working power paralleling the increase in activity seen in the preceding section, and leading up to the peak of working power already shown to occur at oestrus. At this time the follicles have reached a diameter of 7 to 8 mm. The series dated from the 17th to the 21st days contained 16 specimens. All but one of these had follicles measuring 8 mm. in diameter and over. The one exceptional case, which had 6 to 7 mm. follicles, was dated, from the histological examination, at 20 days and this muscle performed more work per minute (5.6 gm.cm.) than any specimen studied. Three

of the last series, although giving the typical oestrous contractions, as far as working power was concerned, belong in the inter-oestrous group.

Discussion. The series of records made at different stages of the oestrous cycle have served to confirm Keye's observations on the types of



Summary of work records

DAYS AFTER OVULATION	NUMBER OF SPECIMENS	GM. CM. WORK PER MINUTE
1	2	5.45
1–2	1	5.1
2–3	4	3.1
3	7	2.3
1–3	14	3.85
4–8	7	0.18
9–15	9	0.2
16–17	9	0.33
17–18 and 18–21	16	2.3 2.9*
	55	

* If 3 very low records omitted.

the contractions of the circular muscle of the uterus. In addition, the longitudinal muscle has been investigated and simultaneous records taken in a number of cases. At every stage the longitudinal muscle contracted more powerfully than the corresponding circular layer. The minor activity of this isolated circular muscle supports the observa-

tion of Wislocki and Guttmacher (10), on the entire uterus of the sow. They found that rings of constriction were usually absent and that peristaltic contractions predominated. Their "very feeble type," seen while the corpora lutea are retrogressing, is quite explicable in view of the slow, low contraction of the isolated longitudinal muscle of this period. But the fact that they found the uterus inactive, as the follicles matured, is surprising, considering the vigor of the muscle strips. They divided the oestrous uteri, whose ovaries possessed follicles 8 to 11 mm. in diameter, into two groups, those in which the corpora lutea in the ovaries indicated a recent ovulation and those whose ovaries showed no signs of recent ovulation. The uteri of the first groups were large and flaccid, they lacked tone and did not contract spontaneously in the bath of Locke's solution. The others were round and gave the impression of having tone. Some of the latter group contracted vigorously, others not at all. The absence of corpora lutea in the ovaries might be due to this being the animal's first oestrous period or to its having passed through a long dioestrous interval. These different types of oestrous uteri were often seen. As far as the activity and working power of isolated strips were concerned, the presence or absence of corpora lutea of a previous ovulation did not seem to be a factor. Some specimens with corpora lutea were very active, as figure 1 of no. 124. However, the large, flaccid type of uteri which they describe invariably had to be discarded.

The activity of both layers and the working power of the longitudinal layer are at the maximum during oestrus and at the minimum from the fourth to the sixteenth day after ovulation, rising again as the corpora lutea degenerate and the follicles ripen. The changing type of the contraction seems to parallel the activity of the corpora, as has already been reported by Keye. Recent unpublished work done in this laboratory by Mr. David Anopolsky suggests that there is a cycle of changes in the muscle fibers, which may account for the types of contraction in the various phases. An examination of the two layers of muscle does not show a sufficient histological difference to explain the difference in their activity when taken from the same specimens.

It is interesting to compare the two layers of muscle in other hollow viscera. Those from the same part of the alimentary tract ordinarily beat isorhythmically, according to Alvarez (15). Trendelenburg (16) thinks that a precise relationship exists between them in the guinea pig's intestine, one contracting while the other is in the phase of relaxation. Gasser (17) holds that in peristalsis in the guinea pig, first the longitudinal muscle of the intestine contracts and then about a half-second later the circular. He found that the pendular movements continued in plexus-free preparations of circular muscle and in agreement with Bayliss and Starling thinks there is a coördinating mechanism involving an interpolated nervous element which brings about the regular peristaltic contractions.

If the same state holds for the sow's uterus, it cannot be made out from this series of observations. We do not have sufficient knowledge of the intrinsic nerve supply of the uterus to judge the extent to which it is a factor in the movements of the two layers of muscle and how far the rhythm is an innate property of the muscle. It was hoped that records from the separated layers would clear up some of the difficulties in interpretation. It is a simple matter to separate the longitudinal layer but, in the non-pregnant uterus, the endometrium adheres too closely to the circular coat to be removed without injury to the muscle. Consequently it was clipped close with fine scissors. No attempt was made to make preparations free of nervous elements. It was thought that perhaps the minor contractions, which are frequently present in the tracings of both muscles might occur in one layer, their appearance in the other being due to the fact that both contracted in the same phase, and caused interference. However, when present in one separated layer, minor waves always showed in the other. The fact that the circular muscle may contract with greater amplitude and rapidity when isolated from the longitudinal muscle suggests that the layers exercise a mutual inhibitory action. The muscle gives little evidence of tone except during inter-oestrus, in contrast to that frequently seen during pregnancy. The muscle of the uterus is probably subjected to far more radical changes than other plain muscle of the body, just as the endometrium passes through a cycle of changes peculiar to the mucosa of this organ alone. Perhaps the changes in the working power and type of contraction which are so pronounced in isolated strips of muscle do not occur in the uterus of the living animal. Nevertheless, they must be in some degree indicators of profound changes taking place in the physiology of the muscle of the reproductive tract.

CONCLUSION

Observations on the isolated muscle of the pig's uterus indicate that the circular and longitudinal layers function differently and that both pass through significant changes in activity and in working power during the oestrous cycle. The work accomplished is greatest during oestrus and least at the time when the corpora lutea are at the height of development.

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STUDIES OF KIDNEY FUNCTION[1]

I. Renal Excretion with Special Reference to Ambard's Laws

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The present study was undertaken to determine the relationships existing between the amounts of substances excreted by the kidney and the concentration of these substances in the blood stream. Furthermore, it was desired to show to what extent such relationships serve as indices of the functional status of the kidney.

This is by no means a new field of investigation. During the last twenty years a number of papers have appeared but the conclusions reached are far from uniform.

The pioneer work was reported by Ambard (10), (11), (12), (13), (14) and his associates in France. These investigations resulted in three generalizations, the so-called Ambard "laws," which are briefly:

1. With urea excretion at a constant concentration, the output varies directly as the square of the concentration of urea in the blood.

Using a single subject, and regulating water intake so that the concentration of urea in the urine was fairly constant, Ambard obtained a series of figures on urea output and blood concentration which approximated this "law" quite closely.

2. With constant blood urea level the output is inversely proportional to the square root of the concentration of the urine urea.

3. By combining the first and second laws, assuming 25 grams per liter as the standard concentration of urea in the urine and interpolating an additional factor for changes in body weight from an arbitrary standard of 70 kilos, Ambard derived a mathematical expression

$$\frac{Ur}{\sqrt{D \times \frac{70}{P} \times \frac{\sqrt{C}}{\sqrt{25}}}} = K$$

where P is the weight in kilos. The normal range of the coefficient, K, is stated as 0.06 to 0.08, although various temporary conditions, e.g., di-

[1] This study is drawn from the dissertation presented by B. S. Walker in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Boston University, June, 1926.

minished water intake, may cause a high value in normal individuals. One persistently high was held to be indicative of impaired renal function.

In considering this work it should be kept in mind that the analytical methods used by the French at this time were of a low order of accuracy. One of Ambard's substantiating observations was based on a determined blood ammonia value of 0.042 gram per liter.

From elaborate studies with other materials Ambard concluded that all of them had the same secretory constant. These obviously artefactual results must be attributed to crudity of analytical methods. Blood analysis is a matter of nice procedure; with the general lack of development of biochemical analytical methods at that time experimental errors were unavoidable.

Ambard expanded the work to embrace so-called "threshold" and "non-threshold" substances. The latter term he applied to any substance which the kidney continues to excrete as long as there be any of it in the blood stream. Threshold substances were those which are excreted by the kidney only when their concentration exceeds a definite and fairly constant blood level, specific for each. Urea and sulfate were considered as typical non-threshold substances.

In considering Ambard's use of these terms, we must remember that they are used by him in a sense somewhat at variance with our present conception. To him, the threshold was a definite entity, comparable to the height of a dam. It was variable within limits, but in all cases its height determined quantitatively the amount and rate of excretion for a given blood level. Even for glucose he believed that he had demonstrated a definite threshold,[2] and that the excretion of glucose by diabetics was conditioned by the height of this hypothetical dam. By the use of phloridzin in dogs, he "annulled the threshold" and under these conditions he found that the rate of excretion of glucose followed the original formula as proposed for non-threshold substances.

Later Ambard admits that the matter of the excretion of electrolytes is a more complex problem than can be settled by the use of the excretory coefficient.

The reception accorded Ambard's generalization was in the main favorable. Chaussin (17), it is true, called attention to the possibility that the observed constancy was the result of mathematical form rather than an accord with an excretory law. The use of square and fourth roots with the more variable factors promotes constancy independent of theoretical assumption. Even so, the "constant" varies 40 per cent in normals.

In this country, McLean (22), (23), (24) seemingly first called attention to the generalization. Recognizing the crudity of the analytical methods used by the French, he repeated the experiments of Ambard, using more

[2] An error reiterated by Jacobsen (20) a few years later.

accurate analytical methods,[3] and came to the conclusion that Ambard's combined formula was substantially correct.

By certain arithmetical manipulation he derived the more convenient form of the so-called McLean index.

$$\text{Index} = \frac{D \times \sqrt{C} \times 8.96}{W \times (Ur)^2} \qquad (2)$$

A normal index is 100 or higher. With this formula McLean found that in 107 normal cases 31 showed indices below 100 but above 79; 3 were below 79. This observation tends to lower the limit of possible normalcy to about 80, the intermediate values forming a neutral zone of presumptive partial loss of function.

Lewis (21) has determined the McLean index in numerous normal and nephritic cases and shows that in 40 cases whose kidneys as far as could be determined were normal, the value of the McLean index ranged from 228 to 76, with about one-fourth below 100 and 2 cases below 80. Several groups of nephritics showed similar deviations. From these results we may draw the tentative conclusion that the McLean index is not strictly accurate as a diagnostic procedure but that in the majority of the cases it is in accord with the facts.

Working quite independently of McLean, Addis (1), (2), (3), (4), (5), (6), (7), (8), (18) and his associates have carried on studies of a fundamental nature on the excretion of urea. Extensive and detailed investigations under controlled conditions showed variations of over 100 per cent in the first and second laws. Applying the combined formula, however, a rough agreement was obtained which Addis (vide Chaussin) attributed to the mathematical structure of the formula. The authors attempted to determine the ratio of urea excretion to blood urea in rabbits and in man under standardized conditions of food and water intake and obtained reasonably concordant results with the former. With the latter scattered results were obtained showing that other factors than blood urea entered into the determination of the urea output. The variations were as great for the individual as for the group, showing that the variables were transitory and physiological rather than permanent and anatomical.

Later papers demonstrated:

a. That the ingestion of large quantities of water after periods of abstinence produced uncorrelated increments in urea output.

b. That the hypothesis of a maximum concentration for urea and other urinary constituents, beyond which the kidney is unable to concentrate further, is untenable.

[3] Urea by method of Van Slyke and Cullen (28) with determination of preformed ammonia, chlorides by the method of McLean and Van Slyke (25).

c. That with copious and rigidly standardized water intake in man a linear relationship could be demonstrated between blood urea concentration and renal elimination of urea.

The use of the ratio

$$\frac{\text{Grams urea excretion per hour}}{\text{Grams blood urea per 100 cc.}} \tag{3}$$

was suggested in the above papers as being suitable for clinical use in the evaluation of renal functional ability. Busch (16) in a detailed study of 20 cases, all of whom had had acute nephritis, found that a lowering of this ratio was the most sensitive test of impaired excretory function of the many applied.

Austin, Stillman and Van Slyke (15) found that the factors of concentration of urea in the blood and in the urine must be taken into consideration. Using dogs they found that the direct ratio between blood and urine urea gave more consistent results than the Ambard ratio. This was only true, however, with unrestricted water intake as with limitation the volume of urine influenced the urea excretion. The transition point they termed the "augmentation limit" and was determined by numerous experiments on the same subject. Correcting for body weight the authors derive an expression for volumes below the limit as follows:

$$K = \frac{D}{B\sqrt{vw}} = 7.5 \pm 3.0 \tag{4}$$

The variability ($\pm$ 40 per cent) of the results is attributed to unknown causes, "nervous and chemical." Ambard's first law is a special case of this formula; the second law is inconsistent with it. To obviate possible errors in the timing of their experiments, the authors recommend a time period based upon the excretion of creatinin, which has been shown to be remarkably constant from hour to hour. The value of the 24-hour amount of creatinin is determined for each individual subject by an actual analysis and the time interval of the experiment calculated from the content of the collection.

The concept of an "augmentation limit" has been further verified by Rabinowitch (27) presenting clinical data on the application of the formula to human subjects.

The experiments of Hewlett, Gilbert and Wickett (19) on the toxic effects of large doses of urea (100 to 125 grams by mouth) on man have been applied by Adolph (9) to the problem of the laws governing excretion. In these experiments the values of blood urea were many times the normal, a maximum of 245 mgm. per 100 cc. of blood being found. By straight line extrapolation Adolph deduced a threshold for urea at the level of 22

mgm. per 100 cc. of blood. The obvious objection that urea is excreted by the kidney at levels inferior to this figure, is ingeniously but not convincingly explained. He derives a similar threshold value of 2.9 grams for inorganic phosphorus in the blood, using data from the experiments of Wigglesworth and Woodrow (29).

From his synthesis of experimental data Adolph draws the tentative conclusion that the mechanism for the excretion of these substances, one an electrolyte and the other not, is the same. The validity of his conclusions is somewhat obscured by the fact that the concentrations studied were far above normal and normal values obtained only by straight line extrapolation.

Before entering on the experimental portion of this study, one or two additional points require brief consideration.

The work of Addis in particular brings up the question of the possibility of other agencies than plasma concentration influencing the rate of excretion.

According to present knowledge, three general factors, each the summation of numerous minor influences are operative. They are *a*, the composition of the blood; *b*, the blood pressure, and *c*, the condition of the kidney.

Under the general heading of the composition of the blood we have the special topic of the present investigation, i.e., the concentrations of individual substances, and also the factor of the degree of hydremia. Since the kidneys are one of the chief regulators of the water metabolism of the body, it is hard to conceive, regardless of the theory accepted, that a change in the amount of solvent excreted would be entirely without effect upon the excretion of the solutes. Partially under this heading would fall the presence of foreign regulating substances in the blood stream, although their influence may be upon blood pressure as well as directly upon the excretory mechanism of the kidney.

The factor of blood pressure under present conditions of human experiment must remain an uncontrolled influence. We can determine the general systemic pressure, but not that in the renal circulation. Local vasoconstrictions and dilatations have been demonstrated in animals but if such local variations in blood pressure and flow take place, they are unrecorded and uncontrolled during our experiments upon the intact human kidney.

The last factor, that of morphological and physiological condition of the kidney, is the one which we wish to evaluate by means of our various renal function tests. This factor seems, a priori, to be less variable than any of the others. It is, however, so thoroughly masked by the concomitant influences of the other widely variant superimposed effects that conclusions in this regard are always doubtful and frequently demonstrably in error.

EXPERIMENTAL. In the laboratory this study has taken the form of a determination of the rate of excretion of urea in a number of normal individuals and in a lesser number of distinctly pathological cases. Simultaneously with the determination of the rate of excretion the level of the substance in the blood plasma has been measured. In some cases the whole blood as well as the plasma was analyzed. For a further index of the rate of excretion, the creatinin output was measured for the same time period.

Urea was chosen for the major test substance from two distinct considerations. First, its excretion has formed the basis of the work of most of the previous investigators, hence the results obtained are directly comparable to their work, and their data may be drawn upon, if found advisable, to complement and supplement our own findings. In the second place, the analytical determination of urea, both in the urine and in the plasma, is capable of being carried out with a considerable degree of accuracy. With the exception of chlorine, the other constituents of the urine offer analytical difficulties and uncertainties which render them less appropriate for exact study.

Human subjects[4] were used throughout, chiefly in order that our results might be directly applicable to the functional testing of human kidneys. The particular type of experiment is well adapted to study in man, since the coöperation of the subject obviates a great deal of the technical manipulation necessary with animals.

The normal subjects were young adults; medical students, nurses and laboratory workers, both men and women. Most of these were between the ages of twenty and thirty, and all were free from any symptoms of disturbed kidney function.

For the pathological series, cases were studied who had undergone various diagnostic procedures and whose functional status was fairly well established. In this manner it was possible to check the tests under investigation with the results of physical examination and a variety of other functional tests.

Experimental conditions. In all cases the procedure was as follows: the subject emptied the bladder completely and the time was noted exactly. After a specific time interval, usually thirty-six or sixty minutes, a sample of blood was taken by venous puncture and immedately oxalated and centrifugated; in some cases a portion of the whole blood also was saved for separate analysis and comparison. After an equal period had elapsed, i.e., either seventy-two or one hundred and twenty minutes from the beginning

[4] It is with the greatest pleasure that the authors acknowledge their basic indebtedness to the volunteer subjects of these experiments. Cheerful and intelligent coöperation under conditions of not inconsiderable personal sacrifice has made this study possible. In equal measure, the authors extend their thanks to the patients who have contributed to the work.

of the experiment, the bladder was again completely emptied and the urine saved for analysis.

The majority of these tests were made on subjects in a fasting condition, in order to avoid the disturbing influence of meals on blood composition and on rate of excretion (see Addis, loc. cit.). A few, however, were made immediately following meals in order to observe the magnitude of these effects, while still others followed the ingestion of test meals of galactose or urea.

Analytical methods. The analyses of the blood and of the urine were made largely by the methods of Folin or by modifications of the same.

The plasma filtrates were made by the method of Whitehorn (30) in which 4 cc. of plasma are diluted with about 20 cc. of water in a 50 cc. volumetric flask. Four cubic centimeters of the Folin tungstate solution are added, followed by an equal volume of the 2/3 N sulfuric acid, and the whole is made up to the 50 cc. mark before filtration. The values obtained in subsequent analysis of the filtrate must be multiplied by 1.25 to correct for the greater dilution. The advantages of this modification of Folin's technique are that it is possible to obtain a larger amount of filtrate from a given amount of plasma, and that the dilution is made more exactly. All solutions and procedures were frequently controlled by check analyses of material of known composition.

In general, determinations were made on the plasma instead of whole blood in accordance with the recommendation of Wu (31) who suggests that more consistent results may be obtained by this method. He reports that urea is very nearly equally distributed between corpuscles and plasma, but sometimes is higher in the latter. His average of a number of determinations is 19.3 mgm. for the plasma and 17.1 mgm. for the corpuscles. A few comparative determinations of urea in whole blood and in plasma in our own experiments gave the results shown in table 1.

Inspection of the above table shows that whereas the plasma is consistently more concentrated in urea than the whole blood, the difference is small and the relationship is not constant.[5]

Data. The data obtained from our series of experiments are shown in tabular form immediately below. We shall refer to these tables in the discussion of the results and shall use the data therein contained in the other tables and graphs which will be used to emphasize particular points.

Table 2 covers the normal cases, which are designated as to subject by an abbreviated name. The hospital cases in table 3 are designated by number only.

[5] With the chloride, Norgaard and Gram (26) find that the whole blood concentration can be calculated fairly closely by assuming constant concentration in plasma and corpuscles.

In table 2 the fasting subjects and those tested after meals are grouped separately.

In table 3 the cases are simply listed chronologically with no attempt at classification. This will be done in later tables where classes will be taken up separately. Special conditions of fasting, test meals, etc., are however, noted with each individual case.

The following table gives a summary of the experiments on pathological cases. Later these cases will be grouped according to degree of kidney impairment and considered separately.

Application of experimental data. We may now proceed to examine the laws of Ambard in the light of such data as have been made available.

Ambard's first law. "When the kidney is secreting urea at a constant concentration, the output varies directly as the square of the concentration

TABLE 1

WHOLE BLOOD	PLASMA	RATIO B/P
8.6	10.2	0.84
12.5	14.9	0.84
10.5	12.3	0.85
18.8	9.9	0.89
14.7	16.3	0.90
11.3	12.4	0.91
14.9	16.4	0.91
16.4	17.6	0.93
13.8	14.4	0.96
12.9	13.3	0.97
11.7	11.9	0.98
12.1	12.4	0.98
16.5	16.8	0.98
19.9	20.2	0.99

of urea in the blood." As already noted, this generalization was based upon a limited number of experiments, and the analytical methods were of a low order of accuracy. Addis (loc. cit.) using human subjects and improved methods, fails to confirm. In our own series of experiments no attempt was made to regulate the concentration of urea in the urine. Nevertheless, as was the case in Addis' work, the urine concentrations in certain subjects agreed fortuitously to within 5 per cent. Taking these few cases, we can apply this law and see if the variations are small or large. The results of these observations are recorded in table 4.

From this table we see that in individuals the "constant" of the first law varies 10 per cent or more, and that in a group of individuals there is a deviation of the values of the "constant" from the mean of more than 10 per cent, although the occurrence of two almost identical values gives

TABLE 2

Normal subjects

S = subject
W = weight in kilos
Cr = rate of creatinin excretion in milligrams per minute
C = grams urea per liter urine
D = grams urea per 24 hours
Ur = grams urea per liter plasma
V = volume urine excreted during time period
T = time period in minutes
K = Ambard coefficient
I = McLean index

S	W	Cr	C	D	Ur	V	T	K	I
				Subjects in fasting condition					
L.	80	1.12	22.5	16.2	0.413	60	120	0.112	51
Dr.	74	0.64	18.5	8.9	0.290	40	120	0.107	55
C.	78	1.18	4.9	27.4	0.330	281	72	0.100	64
Ha.	61	0.64	32.3	7.8	0.309	20	120	0.097	68
L.	80	1.19	18.8	17.4	0.350	77	120	0.096	69
Dr.	74	1.37	12.1	14.8	0.296	102	120	0.095	71
Dr.	74	0.83	8.4	11.0	0.214	110	120	0.087	84
L.	80	1.10	45.7	15.9	0.378	29	120	0.087	84
N.	70	1.26	7.5	40.9	0.410	453	120	0.086	85
D.	83	0.51	3.5	20.6	0.216	296	72	0.084	90
Ha.	61	1.13	18.4	15.0	0.321	68	120	0.083	92
Sm.	67	1.01	7.4	11.5	0.202	131	120	0.079	103
Hu.	54	0.74	11.0	18.1	0.309	73	72	0.078	105
Cr.	69	1.22	23.4	21.4	0.337	76	120	0.073	118
W.	66	1.01	25.1	16.6	0.291	33	72	0.069	132
S.	64	1.26	8.1	13.4	0.199	138	120	0.069	135
Sm.	67	0.93	17.6	15.2	0.246	72	120	0.067	140
Sm.	67	0.87	20.4	15.2	0.246	93	180	0.065	154
S.	64	1.16	19.9	17.2	0.262	72	120	0.064	156
Da.	63	1.11	25.2	28.2	0.348	93	120	0.062	166
W.	65	1.05	11.0	13.7	0.189	104	120	0.061	175
Wi.	54	0.69	4.7	25.1	0.227	265	72	0.060	177
Cr.	69	1.26	27.0	25.3	0.309	78	120	0.059	179
N.	70	1.53	20.9	24.6	0.272	98	120	0.057	195
K.	69	1.10	31.0	21.7	0.280	35	72	0.056	200
N.	70	1.52	13.9	48.0	0.335	287	120	0.056	204
S.	64	1.00	21.4	13.3	0.202	52	120	0.055	210
P.	101	1.32	39.9	27.9	0.266	35	72	0.054	220
Dr.	74	2.05	8.2	56.8	0.296	347	72	0.053	224
S.	64	1.16	27.8	20.4	0.255	61	120	0.053	232
Sm.	67	1.29	13.6	23.1	0.220	118	100	0.052	240
Cr.	69	0.96	31.0	17.4	0.225	28	72	0.051	247

TABLE 2—*Concluded*

S	W	Cr	C	D	Ur	V	T	K	I
Subjects examined one hour after breakfast									
N	70	1.38	8.09	12.03	0.335	124	120	0.128	39
S	64	1.04	3.92	14.1	0.263	300	120	0.107	57
Sm	67	0.83	5.88	7.50	0.184	108	120	0.095	72
N	70	1.33	22.4	27.2	0.449	101	120	0.089	82
S	64	0.96	10.9	17.8	0.304	136	120	0.085	89
Dr	74	1.00	16.6	17.3	0.302	87	120	0.082	94
Dr	74	1.05	8.87	14.4	0.232	135	120	0.081	97
Sm	67	0.78	12.0	9.36	0.202	65	120	0.078	106
L	80	1.32	19.8	24.0	0.326	101	120	0.075	113
Cr	69	1.17	22.3	22.7	0.342	85	120	0.073	119
Sm	67	0.75	10.6	15.9	0.228	125	120	0.070	129
Da	63	1.03	26.2	34.5	0.442	110	120	0.071	129
Ha	61	1.01	19.6	21.2	0.319	90	120	0.069	135
Ha	61	0.88	9.81	13.1	0.201	111	120	0.065	149
Cr	69	1.19	23.8	28.8	0.335	101	120	0.063	163
Da	63	0.82	33.5	24.8	0.352	59	120	0.062	165
Dr	74	0.97	19.6	24.0	0.266	102	120	0.059	181
Cr	69	1.29	25.5	24.8	0.286	81	120	0.057	198
S	64	0.58	18.4	14.1	0.186	64	120	0.051	244
Subject examined immediately after ingestion of 15 grams urea									
W	65	1.02	20.6	31.9	0.54	129	120	0.097	68

the table for the group a semblance of consistency. These results are in accord with those of Addis and the conclusion seems to be warranted that Ambard's first law is not an accurate statement of the relationship of output to blood urea level under the condition of constant concentration of urea in the urine. There is, however, a rough sort of agreement, and it is still possible to consider this law as an approximation, subject to wide deviations.

Ambard's second law. "When, with a constant concentration of urea in the blood the subject excretes urea at variable concentrations, the output is inversely proportional to the square root of the concentration of urea in the urine." As a result of chance agreement of the blood urea values in certain subjects, we are in a position to subject this law to a similar trial (table 5).

The widely divergent results obtained by the use of this expression lead us to the conclusion that it is entirely fallacious and from now on it is left out of the reckoning altogether. In discarding this law we are in agreement with Austin, Stillman and Van Slyke, and also with Addis.

Ambard's third law: The McLean index. The rejection of Ambard's second law and the observation of wide variations in the first law renders

TABLE 3

Hospital cases

In the first column is given the case number, followed by a symbol indicating the conditions of the test: F signifying fasting, 15U meaning that fifteen grams of urea were ingested immediately preceding the test, 30G similarly signifying a test meal of 30 grams of galactose, etc.

W = weight of patient in kilos
C = grams urea per liter urine
D = gram urea per 24 hours
Ur = grams urea per liter plasma
V = urine volume excreted during time period
T = time period in minutes
K = Ambard coefficient
I = McLean index

		W	C	D	Ur	V	T	K	I
B4	F	46	12.2	9.8	0.262	40	72	0.081	97
B5	F	66	2.0	30.7	0.198	762	72	0.065	151
B5	F	66	11.9	29.4	0.240	124	72	0.052	240
B6	F	59	9.1	21.3	0.254	117	72	0.065	151
B6	F	59	12.7	44.8	0.259	176	72	0.042	360
B8	F	46	1.9	10.5	0.140	283	72	0.067	143
B8	F	46	18.0	31.0	0.255	86	72	0.040	390
B9	F	81	13.4	26.8	0.304	100	72	0.074	118
B9	F	81	12.8	16.7	0.278	71	72	0.087	85
B24	15U	81	29.8	75.2	0.529	210	120	0.063	163
B26	15U	71	31.6	43.5	0.653	115	120	0.094	73
B30	15U	61	13.0	51.8	0.452	330	120	0.069	134
B35	15U	69	19.0	28.8	0.538	126	120	0.107	56
B36	15U	115	31.3	43.5	0.549	116	120	0.101	63
B39	15U	73	26.4	3.5	0.848	11	120	0.144	31
B40	15U	45	7.6	39.4	0.572	430	120	0.098	66
B41	15U	64	14.3	39.2	0.462	315	120	0.081	98
B42	15U	53	37.0	51.0	0.67	115	120	0.074	117
B43	15U	57	9.2	43.2	0.450	390	120	0.079	102
B44	15U	48	13.6	54.0	0.568	331	120	0.075	115
B47	15U	65	22.3	39.6	0.744	148	120	0.117	47
B48	15U	52	14.4	21.6	0.512	125	120	0.109	54
B53	15U	56	9.2	73.0	0.452	665	120	0.059	185
B240	F	64	19.8	41.7	0.250	105	72	0.039	420
B244	F	68	2.7	8.8	0.252	163	72	0.146	30
B246	F	67	4.8	15.8	0.349	165	72	0.130	38
B248	F	63	18.2	17.9	0.370	49	72	0.090	79
B249	F	66	7.3	30.4	0.380	207	72	0.091	78
B259	F	47	7.0	14.4	0.319	103	72	0.095	71
B269	F	88	18.7	14.1	0.327	63	120	0.105	58
B348	F	64	22.4	19.8	0.465	74	120	0.103	60
	30G		7.5	12.6	0.417	140	120	0.152	28
B352	F	125	21.2	19.1	0.393	75	120	0.125	41
	40G	155	32.0	26.0	0.328	68	120	0.081	98

TABLE 3—*Concluded*

		W	C	D	Ur	V	T	K	I
B354	F	53	10.7	9.9	0.279	77	120	0.095	71
B355	F	66	11.5	16.0	0.432	132	120	0.127	39
B357	F	62	19.3	22.6	0.394	98	120	0.083	93
	40G		6.1	19.2	0.344	265	120	0.105	58
B358	F	49	6.1	14.5	0.257	197	120	0.080	100
10G	15U		14.9	52.4	0.576	293	120	0.076	111
B359	F	69	13.8	15.8	0.454	89	120	0.132	37
	30G		19.1	23.6	0.584	110	120	0.125	40
B360	F	66	3.6	11.3	0.246	257	120	0.115	50
B362	F	79	3.5	8.9	0.258	215	120	0.154	28
10G	15U		11.8	36.4	0.658	257	120	0.14	33
B363	F	81	21.6	8.9	0.294	34	120	0.11	53
10G	15U		15.8	58.1	0.59	305	120	0.093	74
B364	F	66	2.9	17.8	0.257	510	120	0.101	63
10G	15U		9.7	44.0	0.893	380	120	0.166	24
B369	F	67	12.7	11.1	0.257	73	120	0.089	81

the third law logically invalid, since we have disproved one of its components and cast serious doubt upon the other. It will be recalled that the third law is merely a combined formula uniting the first and second laws into a common expression with a single constant. There are, on the other hand, certain considerations which restrain us from thus lightly casting aside this generalization. The third law has been widely used in Europe (as the Ambard coefficient) and in this country (as the McLean index) for the evaluation of kidney functional status. Neglecting for the time the truth or fallacy of its underlying assumptions, it is interesting to see, using such data as are available, how accurately it functions as a test of renal efficiency. Out of 58 determinations on normal subjects our own results show 20 depressed McLean indices.[6] Of these twenty, 9 are between 80 and 100, which may be considered as a zone intermediate between normalcy and depressed function. The others range between 39 and 80, indicating a loss of function proportional to the difference between the value of the index as found and 100. There is no reason to doubt that the subjects used had perfectly normal kidneys, since no other signs of kidney impairment were found. This indicates, therefore, that in about 19 per cent of normal cases, the McLean index determination shows loss of function of greater or less degree where none exists.

This observation can be confirmed by considering successive determinations of the index on the same normal individual. Taking the subject S.,

[6] The reader will recall that a depressed McLean Index is equivalent mathematically to an increased Ambard coefficient and indicates depressed function according to the proponents of this method of functional diagnosis.

a medical student in good health, whose McLean index was determined seven times within a period of four weeks, the following values were ob-

TABLE 4

SUBJECT	URINE UREA IN GRAMS PER LITER	UREA OUTPUT IN GRAMS PER 24 HOURS	PLASMA UREA GRAMS PER LITER	"CONSTANT"
Dr.	8.4	14.4	0.214	0.057
	8.2	56.8	0.296	0.039
Ha.	18.4	15.0	0.321	0.083
	19.6	21.2	0.319	0.069
Cr.	23.4	21.4	0.337	0.073
	23.8	28.8	0.335	0.062
Da.	25.2	28.2	0.348	0.066
	26.2	34.5	0.442	0.075
Similarly in different subjects having essentially the same urine urea concentration				
W.	11.0	13.7	0.189	0.051
Hu.	11.0	18.1	0.309	0.073
Da.	10.8	15.2	0.256	0.066
S.	10.9	17.8	0.304	0.072
Sm.	10.6	15.9	0.228	0.057

TABLE 5

	SUBJECT					
	Sm	Ha	Dr	N	Cr	S
Blood urea (gram per liter)	0.202	0.321	0.296	0.335	0.337	0.263
	0.202	0.319	0.296	0.335	0.335	0.262
Urine urea (gram per liter)	7.35	18.4	12.10	13.90	23.4	3.92
	12.10	19.6	8.17	8.09	23.8	19.90
Urea output (gram per 24 hour)	11.53	15.0	14.8	48.0	21.4	14.1
	9.36	21.2	56.8	12.0	28.8	17.2
Ratio of outputs	1.23	0.708	0.26	4.00	0.74	0.82
Inverse ratio of square roots of concentrations	1.28	1.03	0.82	0.76	1.01	2.25
Variation	4%	45%	215%	81%	36%	174%

tained: 232, 185, 57, 156, 89, 210, 244. One of these values, according to the usual interpretation, shows marked and serious functional loss; one

is on the borderline; the rest are well above normal. It is worth noting that the low value was obtained on the same day as the preceding high

TABLE 6

CASE	P.S.P.	MCLEAN	DIAGNOSIS
*B4	78	97	Gonad failure
B5	75	151–240	Psychosis
*B6	76	151–360	Epileptoid
B8	61	143–390	Gonad failure, psychosis
B9	65	118– 85	Psychosis
B24	60	163	Eunuchoid
B26	74	73	Thyroid failure
B30	71	134	Thyroid failure
B35	33	56	Myxedema
*B36	40	63	Thyroid failure, possible nephritis
*B39	47	31	Thyroid failure, incipient nephritis
*B40	45	66	Gonad failure
*B41	60	98	Parkinsonian, thyroid failure
B42	58	117	Surgical castrate
B43	48	102	Parkinsonian
*B44	51	115	Thyroid, incipient nephritis
*B47	40	47	Gonad, hypertension, incipient nephritis
B48	44	54	Pituitary dysfunction
B53	61	185	Pituitary dysfunction
B244	49	30	Bilobar pituitary failure, brain tumor (?)
B246	56	38	Pituitary dysfunction
B248	31	79	Pituitary dysfunction
*B259	63	71	Pituitary dysfunction
*B269	46	58	Aborted acromegaly
*B348	52	60– 28	Thyroid failure, nephritis
*B352	42	41– 98	Thyroid failure, nephritis
*B353	32	80–145	Nephritis, syphilis
B354	55	71	Brain pathology
B355	41	39	Idiopathic epilepsy, gonad failure following mumps, anemia
B357	53	93– 58	Anemia
B358	38	100–111	Hysteria
*B359	34	37– 40	Male castrate, nephritis
*B360	52	50	Hypertension
B362	40	28– 33	Gastric malignancy
*B364	39	63– 24	Pyelitis

* The cases designated with a star all demonstrated a definite kidney pathology.

value. When 2 determinations in succession give divergent and contradictory results it casts a serious doubt upon the validity of the index.

The next table groups a few of the pathological cases.

Here, as in the normal cases, we find a substantial agreement. Out of these 22 indices, all of which should be below 100 according to the theory, we find 3 (2 in the same individual) which are over 100, an error of 14 per cent. Again, the same individual shows a large variation, as in cases B-352 and B-353, where 2 determinations made on the same day show markedly differing degrees of kidney impairment.

These observations present the interesting situation of a formula based upon 2 assumptions, one of which is of proven invalidity and the other subject to wide variation, which is itself a fairly accurate, although by no means infallible index of kidney function. It is obvious that the reasons for its partial validity are not intrinsic in the "laws" from which it is derived. Examination of the formula itself (McLean's modification) is necessary to see just what it measures.

$$\frac{(\text{Gm. urea per 24 hr.}) \times \sqrt{\text{Gm. urea per liter urine}} \times 8.96}{(\text{Body weight in kilos}) \times (\text{Gm. urea per liter blood})^2} = I$$

when I = 100 or more in normals.

The factor 8.96 is merely an arbitrary quantity inserted into the formula for the purpose of making the index 100 when the other quantities are such as would yield an Ambard coefficient of 0.08.[7] It does not figure otherwise in our considerations.

Similarly the body weight factor is a correction applied on the assumption that the amount of urea excreted is directly proportional to the body weight. This assumption is hardly justified as the protein metabolism is concerned with but a small part of this quantity.

The factor of the square root of the concentration of urea per liter urine is based upon the second law, which Addis has shown to be invalid, and his conclusion is confirmed by Austin, Stillman and Van Slyke, and by our own observations.

This leaves the ratio

$$\frac{\text{Gm. urea per 24 hr.}}{(\text{Gm. urea per liter blood})^2}$$

which, other conditions being equal, should be a constant. This is only a restatement of Ambard's first law, with the implied provision that all factors tending to cause fluctuations in the rate of excretion must be held constant.

Addis and Drury, in their experiments under strictly standardized conditions (v.s.), found that this ratio was only constant when both terms were taken *to the first power* and varied when the square of the blood urea

[7] Found by McLean to fit the normal better than Ambard's original figure of 0.07.

was taken. Under the conditions of experiment the urine volume was always large and seemingly did not affect the ratio. Other experiments made under ordinary conditions, did not show a consistent linearity.

When the special conditions of high blood urea and excess water are complied with, the ratio D/Ur is, or approaches a constant.

This does not prove that under normal conditions there is such a linear relationship between blood and urine urea. Addis' own data show an approach to a parabolic relationship when the standard conditions were not maintained.

The question then, reduced to its simplest terms, resolves itself into the consideration of the linear or parabolic relationship of concentration of urea in the blood and rate of elimination in the urine. The solution, patently, requires the application of statistical methods of analysis to the available data. This treatment will be described in a subsequent paper. The results of this portion of the study may be briefly summarized as follows:

SUMMARY

1. The several so-called "laws of kidney excretion" have been discussed and their mathematical formulation analyzed.

2. Experiments have been carried out on normal and nephritic subjects to ascertain the degree of validity of these several concepts.

3. The first law of Ambard is found to be only correct within certain rather narrow limits. At best, it may be regarded only as a rough approximation.

4. The second law of Ambard has been found to be completely invalid, thereby confirming the conclusions of earlier investigators.

5. The third law of Ambard—a combination of the first two—in spite of its highly dubious foundation, is shown to offer results which correlate approximately with the known conditions of renal function.

6. Analysis of existing formulae demonstrates that the crucial point is intrinsic in the mathematical formulation of the interdependence of blood urea concentrations and urea elimination.

7. The cause of this seeming contradiction in the application of Ambard's third law is to be sought by statistical analysis of existing data.

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STUDIES OF KIDNEY FUNCTION[1]

II. The Relation of Blood to Urine Urea

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In the preceding paper the authors have considered the validity of the concepts underlying the several mathematical formulae derived as quantitative expressions of the integrity of kidney function. A series of measurements of blood and urine urea and creatinin was made using normal and nephritic subjects. The data conjointly with those of other investigators flatly negative the so-called second law of Ambard, cast grave doubt on the accuracy of the first law and with a most interesting inconsistency show that the third law—based upon the first two and seemingly with a most dubious foundation—gives results which correlate approximately with the known state of kidney function. Analysis of the several mathematical expressions derives this concordance from the single relationship of relative blood concentration and elimination rate. The present paper presents a statistical analysis of our own and other suitable data to the end of determining, if possible, some clue as to the underlying mechanism of urea elimination.

The excretion equation for urea. In the previous paper it was shown that there are two major schools of thought concerning the relationship between the amount of urea in the blood and its output by the kidney. Ambard (8), (9), (10), (11) and McLean (18), (19) with their co-workers, derive an excretion equation of the parabolic type.

$$Ur^2 = KD \tag{1}$$

where Ur is the blood level and D is the rate of output (grams per unit time). Addis and his associates (1), (2), (3), (4), (5), (6), (15) on the other hand, have shown that under certain conditions the relationship more nearly approaches the linear equation,

$$Ur = KD \tag{2}$$

[1] This study is drawn from the dissertation presented by B. S. Walker in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Boston University, June 1926.

and hold that deviations from this relationship are the results of extraneous factors.

It is pertinent to see to what extent our data agree with either of these generalizations.

If we plot the blood urea values of the normal cases as ordinates and and the urinary urea outputs as abscissae, a wide scattering is found indicating the presence of several influences. Statistical treatment offers a means of balancing the positive and negative errors of a large number of cases and deriving an expression for the general trend. In order to secure

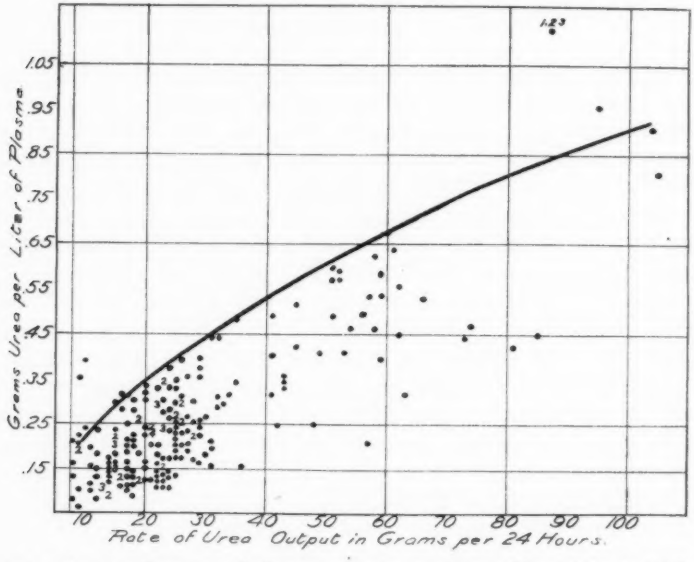

Fig. 1. "Scatter diagram" of all normal cases, as reported by Addis, by McLean, and in our previous paper. The curve is that of Ambard's first law, with a coefficient of 0.10: this curve seems to delimit most satisfactorily the normal area from the abnormal.

a sufficient number of cases to justify the application of standard statistical methods, we have combined with our normal data those obtained from the published work of McLean (18), (19) and of Addis (6) and his associates. Only strictly normal cases have been taken, omitting McLean's normal hospital inmates and those cases of Addis and Drury studied under special conditions of urea ingestion and high water intake. A few cases of urea ingestion have, however, been included where the subject was normal and following a normal routine of daily life. This selection gives data on 220 normal individuals. The distribution of these cases is shown in the ac-

companying plot (fig. 1). That there is some correlation is obvious from inspection of the diagram.

To determine whether or not this correlation is linear, we may proceed, according to the standard statistical method, to the simultaneous determination of the Pearson correlation coefficient and the correlation ratios (22). The calculation of the former involves an assumption of linearity; the correlation ratios on the other hand are equally applicable to correlations involving both linear and non-linear regressions. Hence if the correlation ratios give higher values for a given set of data than does the Pearson coefficient, it may be concluded that the relationship is non-linear.

The Pearson coefficient for the 220 normal cases was 0.840 ± 0.013 and values of the correlation ratios as follows:

$$\eta\, XY = 0.8985 \pm 0.0087$$
$$\eta\, YX = 0.9266 \pm 0.0065$$

In order to avoid a possible error of one set of data overbalancing the others and leading to false conclusions, the Addis cases and those of McLean were then analyzed separately, and in each set the correlation ratios were found to exceed the Pearson coefficient.

From these considerations it is evident that the relationship is not one of simple linearity in normal individuals and under usual conditions of of life. While the determination of the correlation ratios demonstrates the non-linearity of the relationship, it does not give further information as to its nature.

From the diagram (fig. 1) have been taken the averages of all the values of D for a given value of Ur, and these average values plotted on a logarithmic scale. To supplement these data the cases reported by Hewlett, Gilbert and Wickett (16) have been added in order to be able to extend the curve beyond the normal range.[2] Inspection of the logarithmic plot demonstrated that the trend of the points could be represented by a straight line characteristic. The equation of this characteristic is of the form

$$X = AY^b \qquad \text{or} \qquad (\log X) = \log A + b\ (\log Y)$$

where X is the value of the ordinate and Y of the abscissa, while b and A are the parameters determining respectively the slope and the intercepts of the curve.

By substituting the values for X and Y which determine two points on the line in the above equation, e.g.,

$$X = 0.18 \qquad Y = 10$$
$$\text{and}$$
$$X = 2.00 \qquad Y = 278.5$$

[2] These cases represent the data from subjects to whom heroic doses of urea (up to 125 grams) had been administered. The abnormalcy of the experimental conditions precludes a complete authority.

the parameters are calculated as

$$b = 0.72379 \qquad \text{and} \qquad A = 0.0340$$

The final form for the excretion equation for urea is then

$$Ur = 0.0340\ D^{0.72379} \tag{3}$$

$$\log Ur = (8.53148\text{–}10) + 0.72379\ (\log D) \tag{4}$$

Figure 2 shows a comparison of this expression with those of Ambard and McLean, of Addis and Drury, and of Adolph (7).

Within the normal range of blood urea values the equation of Ambard's first law ($K = 0.08$) deviates from our expression by less than the ordinary individual variations from either. At blood urea values higher than 0.70 gram per liter the deviation becomes greater and increases markedly with further increase in the blood urea. It is to this fairly close approximation of the lower part of the curve that we attribute the partial validity of the Ambard coefficient and the McLean index.

The straight line equation of Adolph postulates a urea threshold concentration in the blood, to which he assigns a value of 0.22 gram per liter in one set of data. He explains the continued excretion of urea at blood levels below this figure to a washing-out phenomenon conditioned by the excretion of water. It is in this lower range that our curve deviates most widely from his expression. The washing-out process undoubtedly comes into the problem and is a factor in determining the slope of our curve. It is our feeling that the only justification for Adolph's arbitrary assumption of a threshold for urea is the possibility which it offers of making linear the excretion equation. To attain this simplification, however, it is necessary to make an assumption contrary to every-day experience (that there is a threshold for urea at 0.22 gram per liter) and then explain away the facts which controvert the assumption.

The Addis-Drury line represents values obtained under the standardized régime of high water intake. It may be considered as representing the response of the normal kidney to the abnormal stimulus of excess fluids. It is interesting to observe that if it be extrapolated it passes through the origin of our system of coördinates. This seems again to controvert Adolph's theory of a threshold for urea. *It seems probable that with increased water excretion our curve tends to flatten out and approach a straight line as a limit.*

Our expression is frankly an empirical statement, based upon the averages of a number of normal cases under ordinary conditions. In its determination we have drawn upon the data published by McLean, by Addis, by Hewlett, Gilbert and Wickett, as well as from our own experiments. It is thus a representative expression free from errors of a personal or regional nature as far as these can be avoided in the synthesis of data

from different investigators in different parts of the country. When applied separately to our own data, it becomes a representative line, although the positive and negative deviations are large. Such deviations seem to be quite a normal characteristic of the action of the kidney, and have so far been eliminated in experimental work only by the heroic methods used by Addis and Drury.

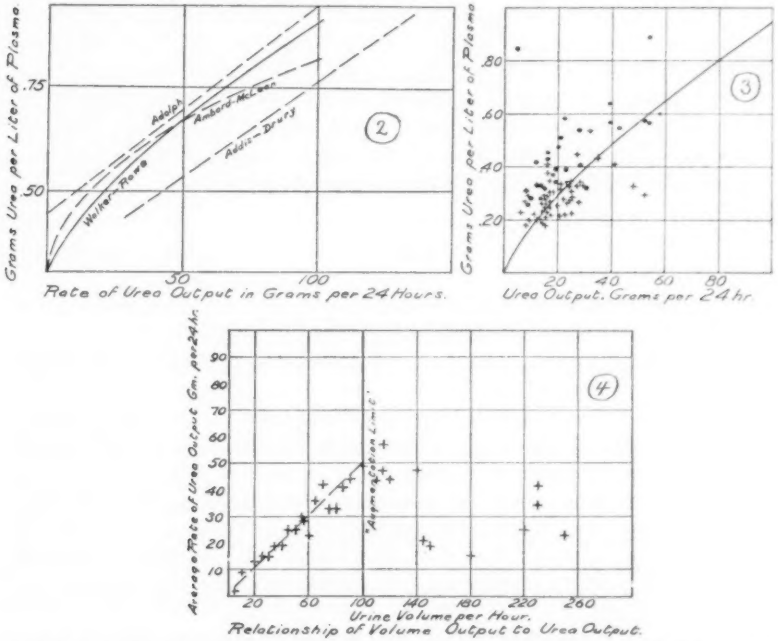

Fig. 2. A comparison of the curve of the derived exponential relationship with those proposed by Ambard-McLean, Addis and Adolph.

Fig. 3. Distribution of the normal and nephritic cases reported in the previous paper with respect to the exponential excretory curve.

Fig. 4. Relationship of volume output by the kidney to simultaneous urea output. Confirmation of the "augmentation limit" of Austin, Stillman and Van Slyke.

APPLICATION OF THE EXCRETION EQUATION FOR UREA IN FUNCTIONAL TESTING OF THE KIDNEY. The next diagram (fig. 3) shows the equation applied as a criterion of kidney efficiency: the normal cases (the authors' data only) are plotted as crosses. Cases of varying degrees of kidney impairment are plotted as circles.

Inspection of the diagram shows that the normal cases are distributed approximately equally upon both sides of the curve. The cases representing kidney impairment, on the other hand, group themselves largely

on one side on the line (21 as against 4). This suggests the possibility of the use of this equation or a similar expression as a means of evaluating renal function. Our number of cases of definite kidney pathology is too small to permit of any specific conclusion. It is of interest, however, to outline briefly the possibilities.

We have shown that over the range of blood urea values usually found, the Ambard equation differs from our empirical expression by less than the normal variations. Our equation being in exponential form renders it difficult to handle, so for the purpose of a first approximation the value of the constant of Ambard's first law may be determined which will include our normal cases and leave out the greater part of the definitely abnormal. By methods of trial and error it is found that with a "constant" of 0.10 in the expression of Ambard's first law

$$\frac{Ur}{\sqrt{D}} = K$$

a curve is obtained which demarcates closely the boundary not only of our own normal cases, but those of the other investigators as well. This curve has been drawn in on figure 1. It includes 97 per cent of the normal cases there represented.

Taking this value of K as an arbitrary limit between the "low normal" and the "abnormal" we may define another limit at $K = 0.075$ between the "low normal" and the "high normal" cases. In other words, if we determine the value of the ratio and find it higher than 0.10, we may regard the subject as deficient in regard to kidney function. If it be less than 0.075 the subject may be designated as normal. Values between 0.10 and 0.075 may be considered as falling into the "low normal" or indeterminate class.

This statement is subject to an objection applying to the original Ambard formula as depressed renal function is indicated by an increase in the ratio, and the scale is entirely arbitrary.

To obviate this, the ratio is inverted and multiplied by 7.5, which gives a value of 75 for the inferior limit of the "low normal" group, and a value of 100 for the superior boundary between this and the "high normal" group. This gives an "index figure" corresponding with that proposed by McLean in which normalcy is represented by an index of 100 or more. Values between 75 and 100 form an intermediate zone of slightly depressed function, which may or may not be indicative of renal disorder. Values below 75 are significant of marked and definite loss of function.

Using this grouping it is found that our subjects are distributed as shown in table 1.

High normals exhibit a 24 to 1 chance that the kidneys are normal. Below 75 the odds are about the same that there is definite kidney pa-

thology. In the low normal group the decision is indeterminate and it is necessary to repeat the test or depend upon other methods of diagnosis.

It is also of interest to compare this simple and frankly approximate test with the McLean index. Taking our own data only, the reader will recall from the previous paper (l.c.) that 19 per cent of our normal cases showed a depressed McLean index and 14 per cent of the renal cases gave a McLean index which was normal or better. By the simple ratio test, only 3.5 per cent of our normal cases fall below 75 and 4 per cent of the pathological cases above 100. It may be pointed out that this comparison is not quite just, since the indeterminate cases which fall into the low normal group have not been taken into consideration. The answer is that these cases are frankly indeterminate, lacking conclusive evidence either of normalcy or of pathology. The factors influencing kidney action vary sufficiently so that two or three repetitions of the test should show with a high degree of probability, to which side of the intermediate group the case belongs. Furthermore, the simple ratio test does not give the false

TABLE 1

Distribution of cases

GROUP	AMBARD	INDEX	NORMAL	RENAL CASES
			per cent	*per cent*
High normal	<0.075	>100	76	4
Low normal	0.075–0.100	75–100	20.5	56
Deficient	>0.100	<75	3.5	40

impression of accuracy which is one of the great disadvantages of the McLean index. Our tables show that all values of the McLean index between 57 and 145 are in reality intermediate, since such values have been obtained from both normal and pathological subjects.

The application of the test is simple. The urine collection is made over a period of one or two hours. Blood is taken at the middle of the period, urea determined in both blood and urine and the square root of the urine urea, calculated as grams per 24 hours, divided by the urea of the blood, calculated as grams per liter. This ratio is multiplied by 7.5 to give the index figure on the scale of 100. If the index be below 75 it is indicative of functional impairment. If the index be over 100 the case is presumptively normal. The values between 75 and 100 are indeterminate, with a suspicion of pathology.

Correction factors of various kinds have been tried on this ratio with rather unsatisfactory results. The substitution of a creatinin excretion period for the time period, as suggested by Austin, Stillman and Van Slyke (12) does not increase the consistency of the results in the majority

of cases, and does complicate the computation of the ratio. Also, in the cases studied, which were adults in good health and not markedly over or under weight, it was found that the correction for body weight added neither to the accuracy or consistency of the ratio.

EFFECTS OF URINE VOLUME UPON RATE OF EXCRETION. The volume of urine excreted is a factor which from a priori grounds we might expect to influence the rate of urea excretion. If we take the same cases from our own work and from the published data in the literature and plot the averages of all cases for each value of urine volume, we obtain the distribution of points shown in figure 4. Up to an output of 100 cc. per hour the urea elimination increases as a linear function, the equation being roughly

$$D = V/2$$

Above this limit the relationship becomes obscured by other factors. It is true that the cases where the volume exceeds this augmentation limit (as it has been named by Austin, Stillman and Van Slyke) are relatively few in number, and that the points outside the line represent at most averages of two or three observations, while those within the limit are derived from much larger groups. This observation might cast doubt upon the existence of the augmentation limit were it not for the results of the experiments of the original proponents who determined this relationship upon individual subjects and observed that at the limit (which varies somewhat for different individuals) the previously rising curve became an horizontal line. This observation has also been confirmed by the work of Rabinowitch (21).

Does this relationship between volume output and urea output per unit time enter into the functional testing of the kidney? In the formulas of Ambard, McLean, and Austin, Stillman and Van Slyke the volume has been taken into account. In many cases it is this volume factor which leads the investigator into error in the evaluation of kidney function by these equations. For example, in one experiment with the subject N., a medical student in good health, the $7.5 \frac{\sqrt{D}}{Ur}$ was 117 which placed him in the high normal group according to the present formulation; but since the urine volume was large and the concentration of urea therefore low, the factor $\sqrt{C}$ in the McLean equation was decreased, causing the index to fall to 85, indicating a moderate degree of kidney impairment which did not exist.

Although later work may indicate the advisability of a volume correction factor, the present problem is to determine the efficiency of the kidney in getting rid of urea from the blood stream, and for this measurement, the simple $\sqrt{D}/\mathrm{Ur}$ ratio offers the advantage of simplicity and directness.

Furthermore, it is quite as conceivable that the urea output influences

the water output throughout the normal range as it is to assume that the water output is the causative factor. Crawford and McIntosh (14) in their recent study of urea diuresis bring out this point by showing that the excretion of water not only parallels that of urea, but also is decreased or increased with changes in the amount of urea given. In patients receiving equal large doses of urea daily the volume output was maintained at an almost constant level. They explain this condition by assuming that the urea in its excretion by the kidney carries a certain definitely fixed amount of water with it.

This hypothesis not only explains in a fairly satisfactory manner the general association between urea output and volume output, but also shows why the association fails in the presence of a large excess of water, since in this latter case considerable water is eliminated by the kidneys in excess of that required to serve as a vehicle for the urea and other solutes.

In case this is the true state of affairs, it becomes evident that the inclusion of a volume correction in the ratio for expression of functional efficiency is unnecessary and inadvisable, since statistically speaking there is a determined volume for the excretion of urea at each blood urea level. In individual cases there is wide variation from this statistical mean, but these variations have not been shown to be functionally diagnostic.

Since their diagnostic value is not demonstrable and since they may introduce serious errors, and has already been pointed out, we feel justified for the present in neglecting these correction factors for differences in urine volume. Nevertheless the fact remains that ingestion of large quantities of water does cause an increase in the rate of urea output, and dehydration of the body causes a decrease in the efficiency of elimination.

The only safe conclusion which can be drawn from this contradictory array of evidence at present is that urea output and volume output have a mutual effect upon each other, the resultant of which, over the normal range of values, is best represented by a linear relationship.

The results of this communication may be briefly summarized:

SUMMARY

1. The writers' own data and those of several other investigators have been subject to statistical analysis.

2. The parabolic relationship proposed by Ambard and confirmed by McLean is shown to be more nearly in accord with all observations than the linear relationship defined by Adolph.

3. The mathematical expression for the curve of elimination has been derived and assumes the form

$$Ur = 0.340\ D^{0.72579}$$

4. To simplify this exponential equation and define an empirical and approximate test for kidney function, certain limits to the constants of Ambard's first equation have been calculated and three zones defined respectively of normalcy, abnormalcy and an intermediate area of indetermination.

5. Based on this, a simple procedure for testing the functional capacity is outlined.

6. The influence of urine volume on urine excretion is studied and the existence of an undefined relationship shown to be probable.

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THE FIRST SECONDARY CHANGE IN PULSE RATE FOLLOWING VERY BRIEF VIOLENT EXERCISE

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It is well known that during a period of only a few seconds of strenuous muscular exertion the pulse rate of the normal man increases greatly, and then falls off after the exercise almost as rapidly to about the normal rate. But if the exertion has been more prolonged, the pulse rate falls off rapidly thereafter for only one or two minutes, and then the change becomes more and more gradual, sometimes requiring an hour or longer to return to normal. The work of Bowen (1903) and of Gasser and Meek (1914) and others showed that the rapid rise in heart rate during exercise is chiefly due to inhibition of vagus influence by sensory impulses, and the first rapid fall in rate after exercise, to the rapid return (or partial return) of vagus influence over the heart. The prolonged acceleration of the heart following longer periods of exercise appears to be mediated by another set of influences—not chiefly by a rise in body temperature (Martin, 1914); nor yet alone by an increase in certain waste products of metabolism or the hormone adrenalin, but in some way by that combination of factors which constitute the changes brought about in tissues and blood by the excessive metabolism of exercise.

For several years in the classroom of the senior author an experiment has been carried out, by students, in the course of which a minute-by-minute determination of heart rate was made for periods of thirty minutes or longer following very short periods of violent exercise. In these experiments it appeared to be the rule that the pulse rate rapidly fell from its high level attained in exercise, almost or quite to normal within two or three minutes; and then, immediately, made a small secondary increase in rate from which the fall was gradual. The appearance of this secondary rise was so striking in the graphic curve of pulse rate changes constructed from data of *some* of these experiments, as to suggest that we might have here in the first rapid rise, and in the first secondary rise, the effect of a separation of the two kinds of influences mentioned above as mediating changes in heart rate due to exercise. Was it possible, in other words, that exercise of such vigor and short duration had been used that the first factor, influencing pulse rate rapidly (because it is mediated entirely

through the nervous system), had passed off before the second set of factors (acting more slowly because more time is necessary in changing the condition of the blood) could mediate their effect? Huang and Klyver became interested in this question, and experiments were planned to determine first whether the secondary rise after short violent exercise would prove to be a constant phenomenon under carefully controlled conditions —and, in case it should prove constant, to determine its explanation, if possible. The experiments were conducted with great care and a very brief statement of the method and the results is here given, although it has not been deemed necessary nor desirable to report more than a summary of some of the data.

Twelve college men (five Americans and seven Chinese) were used as subjects of the experiments. One man was 51 years of age and the others ranged from 20 years to 31 years of age. All were in normal health; but a physical examination of one Chinese man (24 years of age) indicated a slightly enlarged heart and the vital capacity of his lungs was a little below average—although in neither respect was he to be considered as abnormal. He was now quite accustomed to physical exercise; but reported that when a small boy he had been under a physician's care for a time because of "a weak heart."

The "standing run" was the form of exercise used, and three different intervals of time (viz., 5 seconds, 20 seconds and 180 seconds) were decided on for the periods of exercise in the experiments. All twelve of the subjects served in the experiments which used 20 seconds of exercise. In this series are 35 experiments. Most of the subjects served either three or four times in this series. Two men served only once each in the series. Only five of the men served also in the 5-second and the 180-second series, and in both these series each of the five served at least twice. There are 13 experiments in the 5-second series and 10 experiments in the 180-second series. The same two operators (Huang and Klyver) were always in charge. Only one subject was present at a time and the experiments were carried out in a quiet basement room at a room temperature of about 65°F.

The subject exerted himself as little as possible on the way to the laboratory and there he rested in a comfortable sitting position for several minutes. Then one operator (H) counted the normal pulse while the other (K) counted the respirations of the subject at rest for six minutes. After the preliminary counts, (K) instructed the subject to stand and take hold of a rigid horizontal bar before him with both hands. (K) held a stop watch and at his signal the subject did the standing run for the interval of the experiment. In the 5-second and the 20-second series of experiments he ran as hard as he could throughout. In the 180-second experiments he began running at a moderate rate and speeded up gradually.

(K) called to him distinctly, the time every half-minute (as, "half-minute," "one minute," etc). The last warning was given 15 seconds before the end of the 180-second period, and the subject then finished the period at his highest possible speed. At the signal "stop," he dropped back at once into his arm chair, in a comfortable relaxed position and remained

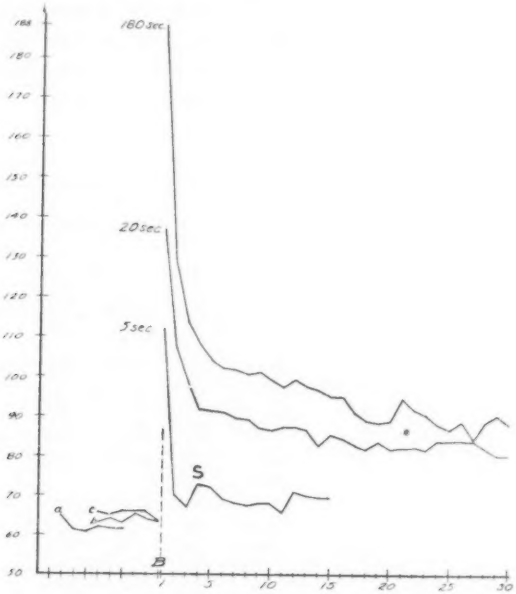

Fig. 1. Pulse rate curves for one subject (Ra) following each of the periods of exercise used.

The curves for the 5-second and the 180-second periods represent the average of two experiments each. The curve for the 20-second period is the average for three experiments. Rate in heart beats per minute is expressed on the ordinates Time (i.e., number of pulse count) is recorded on the abscissa. One count was made during the first 30 seconds of each minute. The first pulse count after exercise is recorded on line *B* in each case. To the right of *a*, *b* and *c* the average pulse rates at rest, immediately before exercise in each series of experiments, are plotted for 6 minutes. Exercise began at the end of curves *a*, *b* and *c* for the 180-second, the120-second and the 5-second experiments respectively.

quiet until the end of the experiment. As soon as the subject was back in his chair, (H) caught his wrist, found the pulse, started his stopwatch with the other hand and began the pulse count. Generally it was possible to begin the first count within four or five seconds after the "stop" signal. The pulse rates before and after exercise were counted in periods of thirty

seconds with intervals of thirty seconds between. It was decided from experience that the 30-second interval was perhaps the best one (better, for example, than a 10-second interval) all things considered, from which to calculate the changing rate per minute. Thus there was one count recorded for every minute. The records and the time were kept at a table behind the subject, where he was undisturbed by them. Noting the second hand of an ordinary watch on the table (H) started his stopwatch at a beat of the pulse and counted mentally. At the end of a 30-second period he stopped the stopwatch and recorded his count silently. The next count was started when the second hand of the watch on the table

TABLE 1

ABBREVIATED NAME OF SUBJECT	NORMAL PULSE AVERAGE	PULSE RATE PER MINUTE IMMEDIATELY AFTER EXERCISE					
		20 second series		180 second series		5 second series	
		Actual rate average	Per cent rise above normal	Actual rate average	Per cent rise above normal	Actual rate average	Per cent rise above normal
Ra	62.8	137.3	218	188.0	300	112.0	178
Ts	73.6	117.3	160	150.0	204	102.0	138
Wu	80.5	132.7	165	174.0	216	119.5	148
Kl	67.0	140.0	209	188.0	280	121.0	181
Hu	78.2	131.5	168	180.0	230	122.3	156
Ge	80.0	160.7	201				
Ka	71.1	128.0	180				
Ph	67.8	121.0	178				
St	66.4	128.0	193				
Ta	68.6	129.3	188				
Wo	65.0	128.7	198				
Sh	73.4	154.0	209				
Average	71.2	134.0	190	176.0	246	115.1	160

came to the position where the last count had been begun. In this way the number of beats per period could be counted and estimated to the half-beat—the result, multiplied by two, gave the rate per minute.

Most of the experiments were carried out between 11:00 and 12:00 a.m. and between 2:00 and 4:00 p.m., and no subject was used for more than one experiment in any half-day.

In figure 1 average curves are given for one subject (Ra) for each of the periods of exercise in order to illustrate the general form of the curve in each case. On line *B* the high pulse rates immediately after exercise are recorded—i.e., the primary or first rise in pulse rate. At *s* on the curve of pulse rate for the 5 seconds of exercise is the first "secondary rise" in

TABLE 2

ABBREVIATED NAME OF SUBJECT	NUMBER OF EXPERIMENT	PULSE COUNTS AFTER EXERCISE BETWEEN WHICH THE FIRST SECONDARY RISE OCCURRED (I.E., MINUTES)	NUMBER OF BEATS RISE OF FIRST SECONDARY RISE	PULSE COUNTS AFTER EXERCISE BETWEEN WHICH THE HIGHEST SECONDARY RISE OCCURRED	NUMBER OF BEATS RISE OF THE HIGHEST SECONDARY RISE
The 5-second series					
Sh	1	2nd and 5th	6	2nd and 5th	6
Ra	2	3rd and 4th	7	11th and 13th	9
	3	3rd and 5th	8	3rd and 5th	8
Ts	4	2nd and 5th	7	2nd and 5th	7
	5	3rd and 4th	3	13th and 14th	6
Wu	6	7th and 8th	1	11th and 12th	4
	7	2nd and 3rd	6	2nd and 3rd	6
Kl	8	7th and 8th	1	10th and 13th	6
	9	3rd and 4th	1	9th and 10th	5
Hu	10	2nd and 3rd	2	5th and 7th	6
	11	2nd and 3rd	7	7th and 9th	13
	12	3rd and 6th	8	3rd and 6th	8
	13	3rd and 4th	8	3rd and 4th	8
Average		3.2 and 4.7	5	6 2 and 8.1	7 07
The 180-second series					
Ra	1	8th and 9th	2	18th and 22nd	10
	2	11th and 12th	2	20th and 21st	10
Ts	3	10th and 12th	5	22nd and 23rd	8
	4	6th and 8th	4	17th and 18th	7
Wu	5	14th and 15th	2	16th and 19th	6
	6	10th and 12th	5	22nd and 23rd	5
Kl	7	9th and 10th	1	24th and 25th	3
	8	8th and 9th	1	16th and 18th	4
Hu	9	6th and 7th	6	19th and 20th	6
	10	7th and 8th	1	29th and 30th	6
Average		8.9 and 10.2	2.9	20 3 and 21.9	6.5

TABLE 3

The 20-second series

ABBREVIATED NAME OF SUBJECT	AGE	NUMBER OF EXPERIMENT	PULSE COUNTS AFTER EXERCISE BETWEEN WHICH THE FIRST SECONDARY RISE OCCURRED (I.E., MINUTES)	NUMBER OF BEATS RISE OF FIRST SECONDARY RISE	PULSE COUNTS AFTER EXERCISE BETWEEN WHICH THE HIGHEST SECONDARY RISE OCCURRED	NUMBER OF BEATS RISE OF THE HIGHEST SECONDARY RISE
	years					
Ra	21	1	5th and 6th	1	23rd and 26th	7
		2	7th and 8th	5	26th and 27th	6
		3	4th and 5th	2	14th and 16th	6
Ts	28	4	5th and 6th	2	15th and 17th	5
		5	5th and 6th	3	12th and 13th	4
		6	4th and 6th	3	28th and 30th	6
Wu	24	7	7th and 8th	1	25th and 26th	4
		8	14th and 15th	2	28th and 29th	4
		9	9th and 10th	4	26th and 27th	8
Kl	27	10	5th and 6th	1	10th and 11th	6
		11	4th and 8th	3	10th and 11th	5
		12	4th and 6th	4	16th and 18th	7
Hu	22	13	8th and 9th	1	15th and 16th	6
		14	3rd and 4th	7	9th and 11th	13
		15	4th and 5th	1	23rd and 24th	7
		16	3rd and 4th	3	15th and 16th	8
Ge	20	17	9th and 11th	6	14th and 15th	8
		18	12th and 13th	3	14th and 15th	6
		19	7th and 8th	1	25th and 27th	14
Ka	23	20	4th and 5th	3	20th and 21st	6
Ph	31	21	4th and 5th	14	18th and 21st	8
		22	12th and 13th	2	24th and 26th	6
		23	6th and 8th	5	27th and 28th	4
		24	8th and 10th	4	27th and 29th	8
		25	4th and 6th	9	4th and 6th	9
St		26	3rd and 5th	7	3rd and 5th	7
		27	3rd and 4th	6	11th and 14th	11
		28	3rd and 7th	6	23rd and 25th	8
Ta	25	29	5th and 6th	2	17th and 18th	11
		30	6th and 7th	3	13th and 15th	6
		31	3rd and 4th	1	13th and 16th	6
Wo	25	32	5th and 6th	2	7th and 8th	4
		33	4th and 5th	2	29th and 30th	8
		34	4th and 5th	3	9th and 11th	6
Sh	51	35	5th and 6th	1	9th and 10th	2
Average			5.6 and 7	3.5	17.2 and 18.8	6.8

pulse rate referred to in case of the very short period of violent exercise—the secondary rise in which we were interested. Further explanation of the figure is given in the legend.

Table 1 gives the normal pulse rate for the individual subjects used, the average rate for each subject immediately after exercise in each series as represented by the first count, and also the percentage of rise over the respective normal rates of each individual subject. The rises, expressed in actual rate, as well as in percentages over the normal, are highest in the 180-second series and lowest in the 5-second series. The average rate as determined from the first counts of all the 180-second runs is 176; of all the 20-second runs 134, and of all the 5-second runs 115. Correspondingly, the average percentage rise in pulse rate over normal in each case is 246, 190 and 160 per cent.

Individual subjects who showed a comparatively high percentage rise of pulse rate in the 180-second series of runs also showed a high percentage rise in both the other series. Out of the thirteen experiments in the 5-second series there are six cases in which the initial rapid drop in pulse rate after exercise went a little below normal, and then the secondary rise, of course, carried the rate immediately above normal. In the other two series of experiments, however, the first rapid drop always came far short of reaching normal. In tables 2 and 3 are gathered data relative to the number of beats increase in the first secondary rise of heart rate and the time of the occurrence of that rise after exercise. It was found that, in the 5-second series, eleven cases out of the thirteen (or 84.6 per cent) had the first secondary rise beginning on or before the fourth count was made after the exercise—i.e., immediately after the first rapid drop in rate. Out of the thirty-five experiments in the 20-second series, sixteen cases (or 45.7 per cent) were found in which this first secondary rise began on or before the fourth count. In the 180-second series no cases of a secondary rise occurred earlier than the sixth count after exercise, while, on the average, the first secondary rise did not show until 8.9 seconds after the beginning of the first pulse count. In the 5-second series the secondary rise began, on the average, 3.2 seconds after the first count—and in the 20-second series it occurred, on the average, 5.6 seconds after the first pulse count began to be taken following the exercise.

The average number of beats rise for the first secondary rise that occurred in the thirteen cases of the 5-second series was found to be 5 beats. For the 20-second series this average rise was 3.5 beats and for the 180-second series it was only 2.9 beats. That is to say, there came a time in all the experiments of each series when a first secondary rise in pulse rate really occurred; but it always came much the earliest in the 5-second series. On the average, it is greatest also in that series and smallest in the 180-second series. If now one observes other later secondary rises in rate in

experiments of the 180-second series (and of the 20-second series) some of these are found to be larger than the first secondary rise in the corresponding experiment of that series. Tables 2 and 3 show the largest secondary rise found in each of the experiments of each series. The average of these highest secondary rises or fluctuations in the 180-second series is 5.9 beats per minute. This is very close (a little greater) to the average rise in case of the first secondary pulse rises of the 5-second series, but not so great, however, as the average of the highest secondary rises in experiments of the 5-second series which amounted to 7.07 beats. After the customary rest in the laboratory the normal pulse of Huang and Klyver was counted in the usual way for 30 minutes. In this time the greatest rise of Huang's pulse was 5 beats and the greatest rise in Klyver's pulse was 6 beats. Likewise, the average greatest variation in pulse rate for all the subjects sitting at rest for the six minutes before exercise in the 58 experiments was 4.7 beats. As will be seen these fluctuations in pulse rate occurred normally (without exercise) under the conditions of the experiment and appear similar to what we have designated "secondary rises" in the pulse in the experiments.

Examining these statements of fact, now, it is clear that in the 5-second series the return of vagus influence is actually complete before the first secondary rise begins, since in that series the pulse rate quickly falls to within the range of normal—often slightly below the average normal for the individual—before there is any secondary rise. Clearly also in the 180- and the 20-second series, the second set of factors—mentioned as mediating their influence through a changed condition of the blood to cause a rise in heart rate—had already begun to exert their influence strongly before the exercise was ended. This is indicated by the very high rise in rate during the exercise and by the fact that the higher rate was more sustained, falling gradually for over eight minutes, on the average, in the 180-second series, and for 5.6 minutes, on the average, in the 20-second series, before any secondary rise showed itself. Moreover, the fact that the average first secondary rise in the pulse rate, as well as the average for the highest succeeding secondary rise, in the "5 seconds of exercise series" are both somewhat larger than corresponding average rises for the 180- and the 20-second series, would indicate that the second set of factors in question *may* be present and concerned to a slight extent in the early secondary rises of the shortest period of exercise. However, the later rather large average secondary rise in pulse rate of the 180-second series and the 20-second series; the similar rises in the normal pulse of Huang and Klyver at rest found in half-hour intervals; and the further similar value of the average greatest variation in pulse rate of all the individual subjects sitting at rest for six minutes; all seem to indicate certainly that the first and very noticeable secondary rise in pulse rate follow-

ing the short (5-second) period of exercise cannot have the full significance first suggested. In this series the rise does occur, separately, after the rise and fall which are dependent on vagus influence, and it comes early thereafter, but its value is comparatively small. Because its value is but little greater than the average normal variation in pulse rate, this early secondary rise of the 5-second series is to be associated not so much with the second set of pulse rate factors, but rather, it must be considered, chiefly, as the beginning of the normal fluctuation in pulse rate which can show itself earlier after the very short than after the longer periods of exercise.

CONCLUSION

The first secondary rise in pulse rate following a brief (5-second) period of violent exercise occurs, on the average, within 3.2 seconds after the exercise, immediately upon the return to normal following the primary rise. Its average value is of nearly the same magnitude as the rises in the normal fluctuations of the pulse at rest and it appears to be chiefly accounted for by the causes that give rise to the normal fluctuations.

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SPECTROPHOTOMETRIC DETERMINATIONS OF PURIFIED BILIRUBIN

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The results of the various investigations which we have carried out with the spectrophotometer (11) leave no doubt that the pigment which is normally found in the gall bladder (4), that which gives rise to jaundice following obstruction of the biliary outflow (7), that which accumulates in the dehepatized animal (3), (4), that which is found in the blood returning from certain vascular areas (such as the spleen and bone marrow) in larger amounts than in the blood going to these areas (5), that which is increased in the body following the injection of hemoglobin (6), that which has been evaluated with regard to the relative amounts formed in the liver, spleen and bone marrow (7), is one and the same pigment.

We believe that this pigment is bilirubin. However, the data on bilirubin as a pure substance are meager. Our knowledge is derived largely from various investigations in which the pigment is mixed with other substances. It is possible, therefore, that the objection might be raised that the pigment we have been investigating is not bilirubin but a closely related substance. While this objection is valid and cannot be eliminated entirely at the present time, it does not invalidate the work reviewed in the preceding paragraph, namely, that the important pigment (whether or not it is bilirubin) found under the various conditions described, is one and the same. Then again, it is possible that this biluribin is a mixture of two substances, which Küster, following the earlier work of Orndorff and Teeple (9) and using ox-gall stones and biliary concrement obtained from a horse, was able to demonstrate. The two substances differ presumably only in the degree of solubility in chloroform and their percentage composition. For the product least soluble in chloroform Küster retains the name biluribin and from analysis of the compound arrived at the formula $C_{16}H_{18}N_2O_3$. Orndorff and Teeple (10) verified the observations of Küster by exhaustive extraction with alcohol before treatment with chloroform. They exhausted the material with large amounts of chloroform, filtered it, distilled off a part of the solvent on a water bath and allowed the liquid to cool slowly. The crystalline product, which separates after standing a

day or two, was filtered, boiled with alcohol and repeatedly recrystallized from large amounts of chloroform. This method gave a perfectly pure crystalline product, entirely free from the material more soluble in chloroform. Orndorff and Teeple make this comment: "Whether the pure bilirubin thus isolated occurs in fresh bile is unknown (10)." From these researches they conclude that analysis of crystallized bilirubin show that the molecular formula for this substance is $C_{32}H_{36}N_4O_6$.

SOLUTIONS OF PURIFIED BILIRUBIN AND OF THE PIGMENT OF BILE AND BLOOD OF NORMAL AND JAUNDICED ANIMALS. Recently we came into possession of a quantity of biliurbin prepared by Dr. H. T. Clarke[1] (department of synthetic chemistry, Eastman Kodak Company) substantially according to the directions of Orndorff and Teeple. In a personal communication to one of us Doctor Clarke says: "I am entirely satisfied with its purity, and there is no question of its identity with the bilirubin described by the above named authors." With this material in hand, we

[1] Doctor Clarke has supplied us with a copy of his comments on the preparation of bilirubin, entered in his notebook at the time of the preparation of the material, some of which we have used in our experiments:

"One hundred grams of beef gall stones (yellow in color) were crushed in a mortar under water and stirred mechanically with cold dilute HCl for several hours. Some carbon dioxide and a little hydrogen sulfide are evolved. The mass is then filtered and the solids washed well with distilled water and finally with a little methyl alcohol to displace most of the water. It is then placed on a fluted filter and extracted first for ten hours with ether, then for twenty-four hours with methyl alcohol (at the end of this time the alcohol takes up no more color), and finally with chloroform, renewing the solvent every twenty-four hours, and continuing until no more color is extracted. The extracts contained well defined crystals of bright orange red color, which appear as rectangular plates under the microscope. When exhausted, the residue on the filter paper is still yellow; it is again digested with cold dilute HCl and the process repeated until very little yellow material remains on the filter. Yield of pure crystalline bilirubin, which is completely crystalline and soluble in chloroform, is 27.0 grams.

"The residue is treated with very dilute sodium hydroxide solution and filtered as completely as possible (this requires several days owing to the colloidal nature of the insoluble portion); the watery filtrate, after allowing to stand over night exposed to the air is acidified with HCl and the dark green precipitate filtered off and washed with water. At the same time all the chloroform mother liquors of the bilirubin are shaken with dilute alkali, freed of chloroform by distillation, filtered and allowed to stand in the air for two or three days. The resulting solution is then acidified with HCl and the dark green precipitate filtered off and washed. The united precipitates are dried at 40°, well ground in a mortar, placed on a fluted filter paper and extracted with ether, which removes a small quantity of a yellowish oily matter. After extraction with ether the residue is extracted with methyl alcohol, after three hours the liquor percolates rather slowly and does not contain a great deal of color. The extract so obtained is poured into an excess of distilled water and collected on the funnel, filtered off and dried at 40°. The yield is 7.0 grams of biliverdin of uncertain homogeneity."

have made comparisons of spectrophotometric data obtained from solutions of purified bilirubin and from similarly prepared solutions of the bile and blood of normal and jaundiced animals.

Bilirubin has weak acidic properties: it dissolves readily in dilute solutions of the alkalies, alkaline carbonates and ammonia. It is insoluble in ether and alcohol; it is insoluble in pure water (since the substance is slightly acidic) but is soluble in water slightly alkaline in character.

In our investigations on the pigment contained in the bile or in sera and plasmas of normal and jaundiced dogs, we have used alcohol-acetone solutions (5). In general, about 30 cc. of blood were taken: the samples were immediately transferred to dry centrifuge tubes and allowed to clot. The clot was then loosened from the wall of the tube and centrifugalized at high speed. Ten cubic centimeters of the serum were placed in a 50 cc. centifuge tube containing 15 cc. of 95 per cent alcohol and 5 cc. of acetone. The tube was then stoppered and shaken well and placed in the ice box for two hours. After again centrifugalizing at high speed the clear supernatant liquid was pipetted into a dry tube for examination with the spectrophotometer. Tests have demonstrated that the solutions prepared in this manner are slightly alkaline.

In the case of the bilirubin sent to us by Doctor Clarke, it was necessary to add a slight amount of a weak solution of sodium bicarbonate or two or three drops of a N/10 solution of sodium hydroxide to the alcohol (75 per cent) acetone (25 per cent) solvent in order to keep the bilirubin in solution.

The purified bilirubin is insoluble in blood serum or plasma of normal or jaundiced dogs but dissolves readily in serum or plasma to which a small amount of alkali has been added. This alkalized serum containing purified bilirubin seems similar to serum obtained from jaundiced animals. It reacts to the direct van den Bergh test and is readily extracted by chloroform. Quantitative recovery of the added pigment may be attained by extraction with the alcohol-acetone solution in the same manner as we have followed throughout our investigations on bile pigment. This fact affords ample proof that bilirubin in the blood may be quantitatively determined by the spectrophotometric method after extraction with the alcohol-acetone mixture.

Moreover, we have observed that pure bilirubin in slightly alkaline solutions is precipitated very slowly when the solutions are made neutral or even slightly acidic in character. After standing for an hour or two such solutions are frequently slightly turbid. In the course of our previous investigations we have had to discard, on occasion, various solutions that were turbid. In a previous paper (11) we noted that this lack of clearness might be due to slight degrees of hemolysis. It is also possible that the factor of slow precipitation of pigment from certain solutions might have

been present and, therefore, one of the causes of turbidity, if not the only one. Hence early in our work we established the criterion that comparisons of amounts of pigment in blood serum or plasma should not be attempted in any set of experiments unless the spectrophotometric readings in the greater wave-length region of the visible spectrum (540 to 700 mμ) were approximately 90 per cent or better and that the readings for all curves in any set of determinations used for drawing conclusions should be comparable to each other in this spectral region. At this point, also, we are willing to grant any critics the truth of the statement that quantitative determinations which depend on very low (10 per cent or less) or very high (90 to 100 per cent) readings of transmission as determined spectrophotometrically may be subject to a fairly high percentage of error. However, this in no wise vitiates the point of argument with which we are concerned nor does it invalidate in the least the conclusions reached in our series of papers on this subject. Furthermore, we would again call attention to the experimental criteria which we have emphasized in a previous paper (11).

COMPARISON OF SPECTROPHOTOMETRIC OBSERVATIONS ON PURIFIED BILIRUBIN WITH BILE AND BLOOD SERUMS OF NORMAL AND JAUNDICED ANIMALS

Pure bilirubin in slightly alkaline solutions gives the direct van den Bergh reaction, or a reaction of the same character as that given by the van den Bergh method when used with bile or blood serums from normal or jaundiced dogs.

Spectrophotometric transmission curves. Several sets of experimental data were obtained on the percentage transmission of light of various wave-lengths through solutions of purified bilirubin of various concentrations. These were compared with similar sets of spectrophotometric observations for various concentrations of bile originally taken from the gall bladder of a normal dog. In all instances the method of preparation of solutions was the same. The alcohol-acetone solutions of purified bilirubin were made slightly alkaline by the addition of a little dilute sodium hydroxide. In general, the solutions of pure bilirubin were made up originally to correspond to 1 mgm. of substance to 1 liter of solvent. In previous spectrophotometric determinations of bile pigment we found that the minimal limit of sensitivity of the van den Bergh reaction corresponded to a reading of about 7 or 8 per cent transmission at wave-length 430 mμ on the Keuffel and Esser spectrophotometer when using containing tubes of 10 cm. length, and we proceeded thereafter to make further dilution of solutions and to obtain spectrophotometric determinations with the reading and degree of concentration just cited as our arbitrary starting point. To get approximately the same reading at the same wave-length with pure bilirubin it was necessary to use 2 mgm. of bilirubin for each liter. In figure 1*A* is shown a group of curves giving the percentage trans-

mission of solutions of purified bilirubin for various wave-lengths. In curve *1*, the concentration was 1 mgm. for each liter: this we have taken arbitrarily as representing a concentration of 100 per cent. In each of the succeeding curves the dilution was increased 10 per cent over the immediately preceding one. Figure 1*B* gives a representative group of curves

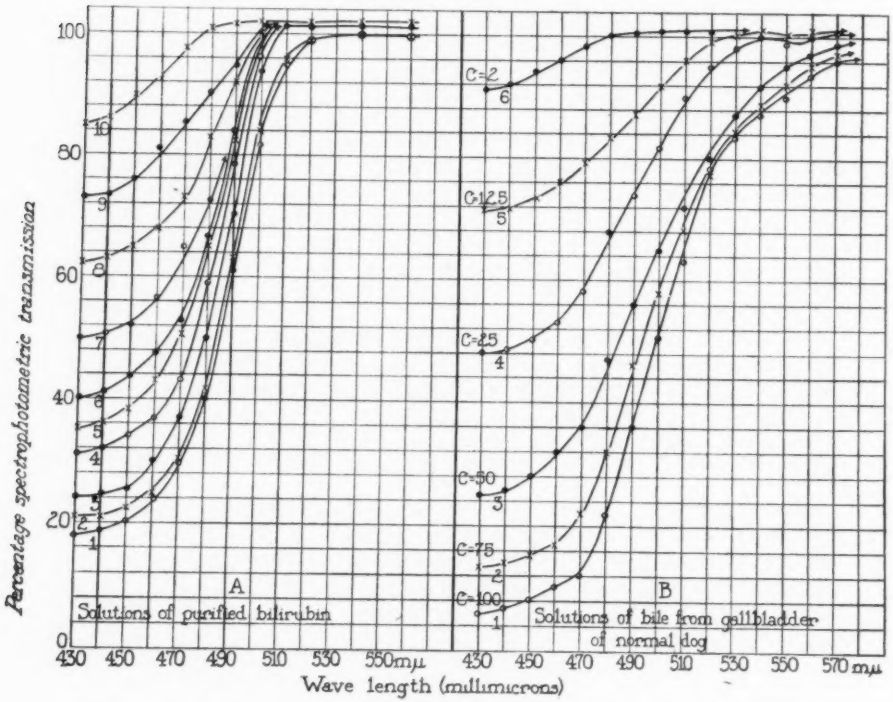

Fig. 1. *A*. Solutions of purified bilirubin. Curve *1* shows the spectrophotometric transmission of a solution of purified bilirubin, 1 mgm. of bilirubin for each liter. Each of the succeeding curves, from *2* to *10* inclusive, is for a further dilution of 10 per cent respectively. *B*. Solutions of bile from the gall bladder of a normal dog. Curve *1*, taken as having a concentration of 100 per cent, shows the percentages of spectral transmission for a solution of bile which is at the approximate limit of accurate test by the van den Bergh method.

showing the percentages of transmission of light for various wave-lengths with changes in the concentration of bile originally taken from the gall bladder of a normal dog. A comparison of these two sets of curves as diagrammed in figure 1*A* and *B* shows that the spectrophotometric transmission curves are comparable in every particular in the two cases. The

spectrophotometric readings for wave-lengths ranging between 430 and 560 mμ only have been plotted: all readings for the range of wave-lengths 600 to 700 mμ were between 95 and 100 per cent. Further comparison of the curves obtained with purified bilirubin should be made with results published in our earlier papers.

Effects of fading, or increase of spectrophotometric transmission with time. In a previous paper (11) we have recorded the effects of fading or the

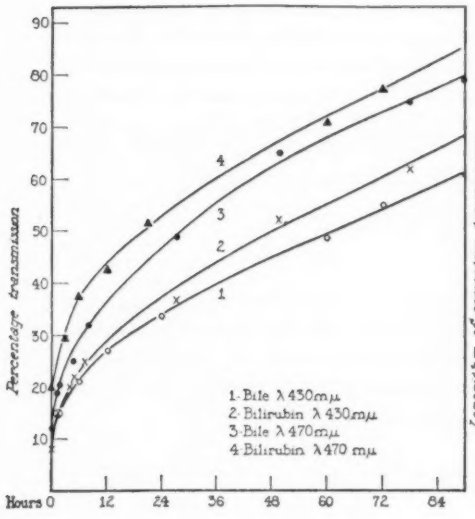

Fig. 2

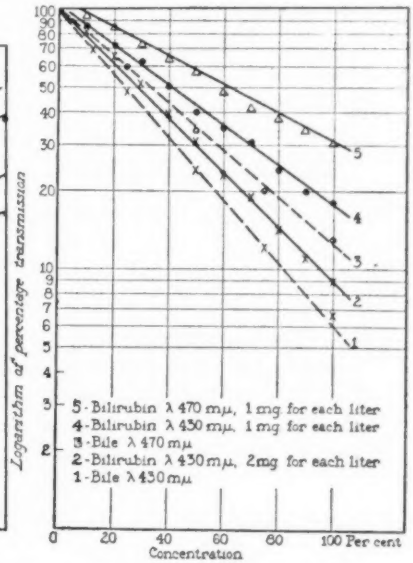

Fig. 3

Fig. 2. Curves showing the fading or increase or spectrophotometric readings in the course of ninety-two hours for solutions of purified bilirubin (curves *2* and *4*) and solutions of bile from the gall bladder of a normal dog (curves *1* and *3*) for wave-lengths 430 mμ, and 470 mμ, respectively.

Fig. 3. Curves showing the results of the application of the laws of Lambert and Beer to the absorption of light by solutions of varying concentration for wave lengths in the absorption zone. Curves *1* and *3* are for solutions of bile from the gall bladder of a normal dog; curves *2*, *4* and *5* are for solutions of purified bilirubin for the wave-lengths specified.

increases of spectrophotometric transmission with time in alcohol-acetone solutions of bile from the gall bladder of a normal dog. In figure 2, curve *1* shows the relationship between the percentage transmission of light for wave-length 430 mμ and the time (in hours) after the removal of the bile from the gall bladder. Curve *3* indicates a similar relationship for

wave-length 470 mμ. Curve *2* shows the results obtained with pure bilirubin (1 mgm. for each liter) for wave-length 430 mμ over a period of ninety-two hours; curve *4* contains the spectrophotometric measurements obtained on the same solution at wave-length 470 mμ. A comparison of these curves definitely shows that there are changes in spectrophotometric transmission which depend on the element of time, and that these changes are analogous and follow the same course in solutions of purified bilirubin and of bile from the gall bladder.

Applications of the laws of Lambert and Beer. It is necessary to establish some standard of measurement in order to be able to compare the amount of light absorbed by one substance or solution with that absorbed by another. For this purpose the extinction coefficient, symbolized by ϵ, was introduced by Bunsen and Roscoe. From Lambert's law, $\epsilon = \frac{-\log I}{X}$ in which I represents the intensity of the light for any given wave-length and X the depth of solution. From Beer's law

$$\frac{C_1}{C_2} = \frac{\epsilon_1}{\epsilon_2}$$

hence the absorption of light by different concentrations of the same substance in the same solvent is directly proportional to the concentration, C. In all of our investigations we have used tubes of 10 cm. length; hence X, the depth of solution, is a constant. Therefore, if the laws of Lambert and Beer are applicable to the data obtained,

$$\frac{C_1}{C_2} = \frac{\epsilon_1}{\epsilon_2} = \frac{-\log I_1}{-\log I_2}$$

In figure 3 we have plotted on semilogarithmic paper the concentrations as abscissae and the percentages of light transmission as ordinates for *a*, various dilutions of bile from the gall bladder of a normal dog for wave-lengths 430 mμ (curve *1*) and 470 mμ (curve *3*) and *b*, various concentrations of purified bilirubin (curves *2*, *4* and *5*) prepared in the manner previously stated. Curve *3* indicates the effects of successive dilutions of 10 per cent each of a solution initially made up to contain 2 mgm. of bilirubin for each liter (marked 100 per cent concentration) and for wave-length 430 mμ. Curve *4* shows the relationship between concentrations of solutions and the logarithms of the transmitted light for a solution, initially made up to contain 1 mgm. of bilirubin for each liter, for wave-length 430 mμ. Curve *5* shows similar data on the same solution as was used in obtaining the measurements used in curve *4*, but for wave-length 470 mμ. For all of these instances, as well as in other tests made but not presented, the alcohol-acetone solutions of bile from the gall bladder and the slightly

alkaline alcohol-acetone solutions of purified bilirubin conform to the law that

$$\frac{C_1}{C_2} = \frac{\epsilon_1}{\epsilon_2} = \frac{-\log I_1}{-\log I_2}$$

CONCLUSION

Various comparative tests have been made on the solubility of purified bilirubin and of the pigment of the bile and of the blood of normal and jaundiced animals. Comparison has been made also of spectrophotometric data on purified bilirubin with the pigment in bile and blood serums and plasmas, of the effects of fading (or changes with time) in these substances in solution, and of the applicability of the laws of Lambert and Beer to solutions of both purified bilirubin and bile pigment. From these comparisons we believe that we have additional evidence in support of the belief that the pigment with which we have been dealing in our series of investigations is bilirubin (1), (3), (4), (5), (6), (7), (8), (11).

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